THE · BACTERIOPHAGE · AND ITS · CLINICAL · APPLICATIONS

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By

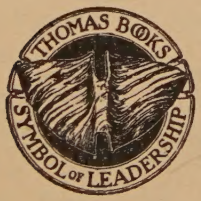
F · D'HERELLE

Professor of Bacteriology
Yale University School of Medicine

Translated by

GEORGE · H · SMITH

Professor of Immunology
Yale University School of Medicine



CHARLES · C · THOMAS · PUBLISHER

SPRINGFIELD · ILLINOIS BALTIMORE · MARYLAND

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Preface

TOWARD the end of the last century physicists and chemists contemplated with pride the superb structure which they had built upon foundations provided by the theories of ether vibrations and of the indivisibility of the atom. For them natural phenomena held few, if any, secrets, and it only remained to explain a few incidental points. A few troubled minds hesitatingly entertained a doubt as to the solidity of the ether which held the foundations in place, despite the fact that its rigidity was as infinite as theoretical, and they suspected that perhaps indivisibility could not be an essential property of a particle occupying a position in space. The physicists merely shrugged their shoulders.

However great the glories of the structure, within but a few years all had fallen to the ground; the theory of ethereal vibrations no longer sufficed to explain the newly discovered phenomena, and the atom proved to be a world of astounding complexity. Like a modern Sisyphus, the physicist, as also the chemist, rolled back the stones to rebuild the edifice, but it still had, unfortunately, only the ether for its foundation.

As the last century closed—that period of blissful

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satisfaction—the biologists also had erected a splendid structure into the foundations of which they had harmoniously interlocked the cellular theory of life, the theory of the fixity of bacterial species and that of the "antibodies" ornamented with "side-chains" such as would explain recovery and all immunity. Suddenly bacteriophagy made its appearance. The structure could not support the added weight of the new facts: it crumbled. The cellular theory of life is manifestly false, for life is an attribute of infracellular particles. The antibodies play no part in the phenomena of recovery. The form and the properties of bacteria are inherently variable characters.

There still remain not a few who struggle desperately to save the ancient theories, who refuse to look squarely at the facts, and who attempt with admirable ingenuity to so adapt the phenomena observed that they will enter into the plan of the outworn theories. Such efforts are in vain.

In the pages which follow an attempt has been made to explain as simply as possible the extremely complicated subject of bacteriophagy. An effort has been made to make the text understandable to all intelligent persons, although it is addressed especially to practitioners of medicine.

The therapeutic applications derived from the phenomena are considered, and it is but just to state that these applications are daily being extended to embrace

more and more diseases and that today in most of the large hospitals of the world they form a recognized mode of treatment.

Each of the chapters of the text corresponds to one of the Lane Lectures, delivered at Stanford University, in October of 1928.

F. D'HERELLE

September 2, 1929

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CHAPTER I

Bacteriophagy

FROM the very beginning of Bacteriology every student of the subject has, at one time or an other, observed irregularities in the growth of bacterial cultures. Noted particularly with bacteria derived from the intestinal tract, and especially in young cultures shortly after isolation, these irregularities have been manifested in several ways. Sometimes the cultures have grown poorly, growth remaining scanty or even disappearing after development had once started. At other times cultures upon agar have become denuded in localized areas; and at still other times the organisms themselves have presented transitory, abnormal characteristics, or have undergone mutations apparently without a directing cause. Examination of the literature reveals the fact that thousands of such observations have been mentioned, but in each instance the author has done no more than to record the isolated fact. No one has established a correlation between all of these strange phenomena, nor has anyone sought to determine their cause and their significance. Indeed, no one seems to have suspected that these diverse manifestations were

but expressions of a general phenomenon which dominates all Bacteriology and indirectly modifies Pathology, Immunology, and Hygiene.

In 1908 I had the opportunity to observe, in Mexico, a very contagious disease affecting locusts, and from the organs as well as from the excreta of the affected insects I isolated a coccobacillus, cultures of which reproduced the disease in healthy locusts. At the request of different Governments I conducted experiments, throughout several years, upon the destruction of these insects. During these studies it was found that under artificial cultivation the virulence of this coccobacillus quickly became attenuated. This made it necessary to make frequent passages from locust to locust, and at every passage the coccobacillus could be isolated from the diarrheal stools. In the course of these investigations the cultures frequently presented irregularities, consisting principally of sterile areas scattered throughout the growth upon the agar. This observation interested me greatly, but I could not succeed in reproducing the phenomenon at will.

Impressed by the work of de Schweinitz and Dorset on hog-cholera I at first thought that the disturbing element was an infravisible, associated virus which was the direct cause of the disease and which, upon the agar medium, interfered with the growth of the coccobacillus, an associated organism. This hypothesis

led me to make many filtration experiments with porous filters, with a view to obtaining the suspected ultravirus in a pure state, and I attempted to reproduce the disease by the injection of these filtrates into healthy locusts. The results were highly inconsistent, but each time that I succeeded in reproducing the disease I recovered the coccobacillus from the dead insect, even though this organism had not been inoculated. I have learned since that when the injected filtrates induced the disease it was because of filter-passing forms of the coccobacillus, but at that time I believed that the disease could be caused only through the simultaneous presence of a filterable virus and the coccobacillus, the latter occasionally being found in healthy locusts. I then turned to experiments involving the simultaneous inoculation of filtrates and cultures of the coccobacillus.

These experiments were interrupted by the war and I became engaged in the preparation of vaccines for the Allied Armies. Thinking that I might be able, perhaps, to encounter similar phenomena in human intestinal diseases I continued the studies with the stools of dysentery and typhoid patients, mixing filtrates of the stools with cultures of the specific bacilli and injecting such mixtures into guinea pigs and rabbits, hoping thereby to reproduce the disease in them. One day in the course of these experiments, having mixed a quantity of a filtrate, obtained origin-

ally from the stool of a dysentery convalescent, with a culture of dysentery bacilli, I placed them in the incubator, hoping thus to obtain a sort of "ripening,"—a more intimate association between the two organisms. What was my astonishment on the following day to find the culture media perfectly clear. Immediately it was apparent that I had been following a false trail; that there was indeed an ultravirus in the filtrate, but that this ultravirus was not pathogenic for the man or the animal but rather for the bacteria. In them it must provoke a contagious disease leading to their dissolution. From then on I was upon the right track, as confirmatory experiments beyond number have demonstrated, and I was thus led to conduct a series of investigations, the results of which I wish to present in the following pages.

Let us first reproduce the fundamental experiment. Let us take a few drops of stool derived from a convalescent from bacillary dysentery. Let us emulsify this in about 20 cc. of sterile bouillon and filter it through a porous porcelain filter, such as the Chamberland, or through a silica candle such as the Berkefeld. Let us add to a young bouillon culture of the dysentery bacillus a drop of this filtrate, and place the tube in the incubator. At first the bouillon appears cloudy, but after a few hours we note that it becomes more and more clear, and, finally after about 12 hours, sometimes more quickly, it becomes per-

fectly limpid. At this time all of the bacilli are dissolved.

It is permissible to conclude from this experiment that there was present in the stool of the dysentery convalescent a principle having the property of dissolving the specific organism which causes the disease.

But if we would accept the idea that this destroying principle is an ultravirus which provokes, in bacteria, a disease leading to their dissolution we must adopt a further deduction. The phenomenon must be transmissible in series, for the destroying principle must reproduce itself after the fashion of all parasites. Let us take, then, a new, fresh culture of dysentery bacilli and add to it a drop of the limpid fluid which remains after the disappearance of the bacilli from the first mixture. Let us place this second tube in the incubator. We will find that the phenomenon repeats itself, for after a few hours all of the bacilli are again dissolved and the liquid is clear. We may then remove a drop of the second dissolved culture and introduce it into a third culture of dysentery bacilli. Once more the phenomenon of dissolution takes place.

One might in this manner continue the passages indefinitely, introducing into each new, fresh culture of dysentery bacilli a drop of the preceding one after all of the bacilli have been dissolved. Far from diminishing in intensity in proportion to the degree of

dilution of the initial drop of filtrate the phenomenon becomes, on the contrary, more intense. Thus it is that after more than a thousand successive passages I have been able to obtain the complete dissolution of the 2,000 million bacilli contained in 10 cc. of bouillon, by adding the infinitesimal quantity of a billionth of a cubic centimeter of the preceding dissolved culture.

Such experiments demonstrate that the principle which destroys the bacteria, and to which I have given the name "Bacteriophage," reproduces itself in the course of its action. The phenomenon of bacteriophagy consists essentially, then, in a dissolution of bacteria under the influence of a principle which reproduces, the latter phenomenon, that is, reproduction, being directly related to the bacteria which are dissolved.

Various questions now arise. Is this bacteriophage found only by chance in the intestinal tract of certain dysentery patients, or is it a constant phenomenon? We will return to this question later. Is the phenomenon of bacteriophagy limited to the dysentery bacillus? I have been able to establish the fact that bacteriophagy is a general phenomenon. It has been possible to isolate races of bacteriophage leading to the dissolution of bacteria belonging to very varied species, such as *Eberthella dysenteriae, paradysenteriae, typhi, paratyphi,* and *sanguinaria; Escherichia*

coli; Salmonella schotmülleri, pullora, suipestifer, and *typhi-murium; Proteus vulgaris; Vibrio comma; Pasteurella pestis,* and *bovis; Corynebacterium diphtheriae;* and *B. subtilis.* Other investigators have isolated bacteriophage races active with staphylococci, streptococci, and pneumococci, and even with bacteria parasitic of plants, such as *Rhizobium radicicolum, B. tumefaciens,* and *B. carotovorus.* The diversity of the bacteria attacked warrants the belief that the phenomenon is, indeed, general, perhaps involving all bacteria.

The problem which next confronts us is to delimit the conditions governing the phenomenon, and this I have undertaken to do.

The media in which bacteriophagy best occurs are those having a pH between 7.2 and 8.2, an alkalinity which corresponds to the culture media employed in bacteriology.

The question of the condition of the bacteria is much more difficult to solve, and for this we will see the reason. Indeed, it is the subject of a debate which is not yet terminated. From the beginning of my studies I have stated that only living bacteria undergo bacteriophagy. Upon this everyone seems agreed. A very simple experiment, however, shows that under certain conditions dead bacteria may be dissolved, although this process is not comparable in all respects to the dissolution of living bacteria. Let us take a

bouillon culture of Shiga bacilli 48 hours old and let us dilute it with sterile bouillon in such a way as to obtain a medium containing 300 million bacilli per cubic centimeter, the count being made by the Wright method. On the other hand let us make a count of the living bacilli, those capable of reproduction, by planting measured quantities of the culture upon agar plates, counting the colonies after incubation. We will find, if we average several experiments, that only 125 million out of the 300 million bacilli are living. To 10 cc. of this suspension, containing 300 million bacteria per cubic centimeter, let us add a drop of a very active bacteriophage fluid. Within a few hours we will obtain a complete dissolution. From this it appears that not only have the 1,250 million living bacilli present in the 10 cc. been destroyed and dissolved, but also the 1,750 million dead bacteria. Whatever may be the interpretation that one chooses to give to this experiment it is none the less true that the dissolution involves both the living and the dead bacterial cells.

Early in my studies I also stated that young bacteria were most readily attacked. This fact all authors have recognized, but Bordet, and, following him, all of those who have adopted his theories, have gone further and have sought to show that the only bacteria susceptible to bacteriophagy were those in process of division. All of the theories of Bordet are

founded upon this affirmation. The preceding experiment, which can very readily be verified, already shows that this statement is incorrect. Furthermore, many authors have furnished experiments which demonstrate that old bacteria, those certainly no longer able to undergo division, are dissolved perfectly. I might cite two such experiments. The first is that of a collaborator of Bordet, Gratia, who, working with Rhodes, obtained complete bacteriophagy of staphylococci eight days old, when a large number of the cocci were already in process of degeneration, and, according to the observations of many students, certainly beyond the state of reproduction. Incidentally, Bordet has never called attention to this experiment performed by his collaborator. It strikes directly at the basis of his theories.

Another particularly conclusive experiment has been performed by Schultz. He caused a bacteriophage weakened by the action of trypsin to act upon a culture of staphylococci and observed dissolution to occur after a delay of from 48 to 72 hours. This author concluded "The particular interest is that such heavily clouded, unlysed cultures may begin to show complete lysis 48 to 72 hours later. In other words, these cultures do not begin to show clarification until long after the period of most active bacterial growth has passed. The peak of bacterial growth is probably attained well within the first 24 hours. These ob-

servations certainly do not harmonize with the concept that the d'Herelle phenomenon is necessarily closely linked with the period of logarithmic bacterial growth."

From these experiments it may be concluded with certainty that, although it may be true that young bacteria are more susceptible to attack, cellular division is not one of the essential conditions of bacteriophagy. The phenomenon does not coincide with the period of division; it may occur with old bacteria already in process of degeneration; and even dead bacteria may be completely dissolved in the course of the reaction.

Up to the moment we have considered only the aspect of bacteriophagy in liquid media. Let us now observe what takes place when it occurs upon the surface of a solid medium. Let us take a series of 12 tubes each containing 9 cc. of a bouillon culture of Shiga bacilli of from 18 to 24 hours old. Let us introduce into the first tube 1 cc. of a *very active* bacteriophage fluid. After thoroughly shaking let us remove 1 cc. from this tube and introduce it into the second tube. After shaking the second tube let us transfer 1 cc. into tube number 3 and let us continue in this same manner until tube number 12 has received 1 cc. from tube number 11. We thus have a series of 12 tubes all containing the same quantity of bacteria, since they were all prepared from the

same culture of dysentery bacilli, together with decreasing quantities of bacteriophage. In tube number 1 the bacteriophage fluid is diluted 1 to 10; in tube number 2, 1 to 100, and continuing throughout the series the dilution for each tube is 10 times greater than that of the preceding tube and the quantity of bacteriophage is one-tenth as great.

Immediately after this operation let us remove 0.1 cc. of material from each tube and let us spread the 12 specimens individually upon the agar of a Petri dish. We will thus have 12 dishes corresponding to the 12 tubes.

Both the tubes and Petri dishes should be placed in the incubator at 37° C., and after incubation the following will be found. Let me state, parenthetically, that the figures given are the average of several experiments. It is well known that in experiments of this type involving large numbers, the figures obtained in a single experiment always deviate more or less from the theoretical figure, which can be ascertained only by securing an average based upon a large number of experiments.

After incubation the first 10 tubes of the liquid medium are clear. Bacteriophagy is thus carried through to completion by a thousand-millionth of a cubic centimeter of the bacteriophage fluid. As for the last two tubes, they are as turbid as a control tube of culture, and experiment reveals that they con-

tain an absolutely normal culture of Shiga bacilli. The action of bacteriophage is thus all or none; intermediary degrees are lacking. As to the Petri dishes, the first 4 are bare. There is no growth whatever upon the agar, or at most but scattered fragments of culture appear. On Petri dish number 5 the fragments of culture are somewhat more abundant. On dish number 6 will appear a layer of bacillary growth seeded with about 100 round spots of very sharply defined contour where the agar is completely free of growth. Petri dish number 7 will show about 10 spots, comparable to the preceding, although somewhat larger. Dish number 8 is covered with a culture of Shiga bacilli and reveals but a single spot. The remaining Petri dishes show only a normal culture of the bacilli.

This experiment immediately suggests that the principle which leads to bacteriophagy must be constituted of active corpuscles suspended in an inactive fluid. At each of the points upon the agar where, during the spreading, a corpuscle was deposited, bacteriophagy takes place, the corpuscle multiplies at the expense of the bacilli in its immediate vicinity, at the same time destroying them so that ultimately each bare spot, which I have termed a *plaque,* represents a colony of bacteriophage corpuscles.

Let us verify this hypothesis. Let us take the seventh Petri dish of the series, where the plaques are

well separated, and with a platinum wire touch a plaque at several points, taking care lest it be touched near the margin. Each time after touching the plaque wash the wire off in a tube of sterile bouillon. We will then find that a trace of this bouillon, introduced into a tube containing a culture of dysentery bacilli, suffices to lead to the phenomenon of bacteriophagy. This is proof that the surface of the plaque is covered with bacteriophage. On the other hand, let us remove, with a loop, a portion of the bacillary culture between the plaques, taking care lest a plaque be touched. Suspend this loopful of culture in sterile bouillon and filter this bouillon through a Chamberland or Berkefeld candle, which, as we have seen, are permeable to bacteriophage and impermeable to bacteria. Experiment shows that this filtrate is completely inactive, since any quantity of it whatsoever is incapable of leading to bacteriophagy. There is, therefore, no trace of bacteriophage between the plaques. These experiments demonstrate in a conclusive fashion that each plaque actually represents a colony of bacteriophage.

We can now understand the significance of the dilution experiment. If there is only one plaque on Petri dish number 8 it is simply because the tenth of a cubic centimeter of culture from tube number 8 spread upon this Petri dish contained but a single bacteriophage corpuscle, and if we make a calculation

based upon the degree of dilution we will find that the cubic centimeter of bacteriophage liquid introduced into tube number 1 must have contained 1,000 million corpuscles. If this is the case, bearing in mind always the dilution, we find that 10 corpuscles were introduced into tube number 9, one into tube number 10, and none in tube number 11. We now see why bacteriophagy was complete in tube number 10 and lacking in tube number 11. A single corpuscle provokes the phenomenon.

And, finally, here is an experiment which affords absolute proof of the corpuscular state. Let us add to 39 cc. of Shiga culture 1 cc. of a dilution of a bacteriophage fluid, which, calculated by the plaque method already described, should contain three corpuscles. Let us distribute the 40 cc. among four tubes, 10 cc. to each. After incubation we will find that three of the tubes are clear, one is turbid. Each one of the three clear tubes has received one corpuscle, the fourth tube has received none.

All of the preceding experiments must be made with very active races of the bacteriophage for, as we will see, races of bacteriophage of very diverse potencies exist and, if a weak bacteriophage is utilized the phenomenon may be masked by the development of a secondary culture of those bacteria which have quickly acquired a resistance to the action of the bacteriophage.

We possess, then, a method which permits us to count the number of bacteriophage corpuscles present in a fluid. This can be done either by counting the plaques to develop on agar after inoculation, or by utilizing the method of dilution, and the fact that counting is possible permits us to penetrate more deeply into the nature of the phenomenon.

Let us bring about bacteriophagy in a bacterial culture and make counts every 15 minutes during the progress of the action. We will find that during the first hour no increase in the number of corpuscles takes place. Then, suddenly, the number increases in the proportion of from 10 to 30 for each one present at the beginning. After a further period of from 45 to 60 minutes a second increase of from 10 to 30 for each corpuscle can be observed. The increase then becomes more and more rapid and attains its maximum at that time when the dissolution of bacteria likewise reaches its maximum.

Let us repeat this same experiment with a very large quantity of culture, 200 cc. for example, by removing, every 15 minutes, a 10 cc. specimen. Let us centrifuge each of these samples quickly and let us make counts of the corpuscles present in each of the supernatant fluids. We will find that at the beginning there is a disappearance of corpuscles from the fluid, but at the end of about 1 hour they reappear and are present in far greater numbers than at the

beginning. Then again their number diminishes definitely only to increase once more after from 45 to 60 minutes. On the other hand it can be shown that the corpuscles which disappear from the fluid are to be found in the bacterial sediment. From these facts it is apparent that the first act of bacteriophagy consists in the fixation of corpuscles to susceptible bacteria. Indeed, Kabelik has demonstrated that the corpuscles exhibit a positive chemotaxis for susceptible bacteria.

From these diverse experiments it seems highly probable that each corpuscle unites with a bacterium and reproduces at its expense; the bacterium then becomes dissolved, liberating the young corpuscles which proceed to attack other uninvaded bacteria, and the same process continues until all susceptible bacteria have disappeared.

Observation of bacteriophagy under the microscope confirms these conclusions. One may see the bacteria swell, some of them assuming a spherical form. Then, suddenly, a process of bursting, lasting but a fraction of a second, occurs and at the point where one formerly saw the swollen but definitely delimited bacterium, there appears only a slight floccule without definite contour. This quickly disappears. Recently Bronfenbrenner has been able to record the process by cinema, and his film confirms the observations which I recorded in 1919. In this connection it is strange

to note that Bronfenbrenner, after having explicitly stated that the destruction of the bacterium is so abrupt that one exposure of the film shows the cell sharply delimited, while it has completely disappeared on the exposure taken 2 seconds after the first, denies that destruction takes place generally by sudden rupture. The history of the controversies which have occurred in connection with bacteriophagy presents, indeed, not a few such contradictions.

Apparently there can be no question but what the bacteriophage corpuscle unites with the bacterium, penetrates the latter, and multiplies there at the expense of the bacterial substance which it modifies and dissolves by means of enzymes which it secretes. This process of dissolution is necessarily accompanied by molecular derangements of the bacterial substance involving an endosmosis and a swelling of the bacterial cell up to the point where rupture occurs. In another chapter we will see that the secretion of lytic enzymes by the bacteriophage corpuscle is not merely an hypothesis, but is an experimentally demonstrated fact.

I have carried out filtration experiments through filters of different degrees of porosity in order to determine the relative size of bacteriophage corpuscles, and I have found that their magnitude is about that of the micella of serum globulin. Various authors, chiefly Prausnitz, and later Bechhold, have repeated

these experiments, taking for purposes of comparison colloid particles of known size, and they have succeeded in determining the diameter of the corpuscles to be between 20 and 35 millimicrons. Bechhold has even succeeded in staining the corpuscles and in photographing them. It is not without interest that he has repeated this work with the corpuscles of smallpox vaccine, and has found that with these the diameter closely approximates that of bacteriophage corpuscles. A number of bacteriologists have found the dimensions to be far smaller, but possibly the results of Bechhold and Villa can well be considered authoritative, for no one would question but that these two physicists, the first of whom especially is universally recognized as an authority in the matter, are infinitely more competent than is a bacteriologist who, without any specific technical training, undertakes experiments upon ultrafiltration. It might be considered somewhat presumptuous for a bacteriologist to contradict the result of a physico-chemical measurement made by a Bechhold.

Bronfenbrenner has recently advanced the hypothesis that the bacteriophage is a soluble product, absorbed by the granules present in the fluid. All experiments refute such an hypothesis. In the first place, we might observe that it is peculiar that only those granules about 20 to 30 millimicrons in size should be the ones, to the exclusion of all others

present in the medium, capable of adsorbing the soluble principle. Furthermore, I have performed many filtration experiments with very dense filters which hindered the passage of corpuscles. Through such an impermeable filter I have first filtered material containing a suspension of bacteriophage corpuscles which remained adherent to the membrane. I have next washed these corpuscles by passing through the filter, either water to which was added hydrochloric acid or sodium hydrate in such concentrations as to provide a pH range between 3 and 9, or bouillon which varied in pH from 7 to 9. In no instance was it possible to obtain a filtrate having, in any degree, the property of inciting bacteriophagy. In this connection the experiment described earlier may be recalled, where it was shown that on agar the bacteriophage is to be found solely in the plaques, even if there is but a single plaque and although there is no trace of it in the bacterial culture between the plaques. How can we explain, then, in this last case, why all of the soluble product should be concentrated on a single one of the granules present in the culture spread over the agar with none remaining in the liquid nor any being fixed to the other granules or to the bacteria present in the culture?

One other proof might be mentioned. Experiments to be described shortly show that one often finds in a single stool two races of bacteriophage pre-

senting different characteristics. Why should we suppose that in such a filtrate *all* of the soluble principle corresponding to one bacteriophage should become fixed on a certain number of granules and all of that corresponding to another race should be fixed to different granules? A mixture must be present, since it is only necessary to derive the bacteriophage from each of the two types of plaque to obtain thenceforth each of the two races in a pure state. I believe that this is sufficient to demonstrate the weakness of Bronfenbrenner's hypothesis.

I have stated that bacteriophage corpuscles possess a negative electrical charge. This statement has been contradicted by several authors, but recently Todd has conducted some very exact cataphoresis experiments and has definitely confirmed the fact that the charge is always negative.

Let us continue the study of the intrinsic properties of the bacteriophage corpuscle so that we may learn something of its biological nature. Let us return to the plaques.

If we photograph a plaque, that is to say, a colony of bacteriophage corpuscles, at the very beginning of its development, we will find that it takes form when the bacteria commence to multiply and when they present but an extremely thin layer upon the agar. The plaque ceases to expand when the culture film increases to such an extent that several layers of bac-

terial cells are superimposed upon each other. One might ask why the plaque does not continue to increase indefinitely until all of the bacteria have been destroyed and why it does not end by spreading over the entire surface of the agar. Let us first observe that bacterial colonies behave in exactly the same fashion as do bacteriophage colonies. Colonies of bacteria do not increase indefinitely but remain limited, and for a given bacterial strain colony size is a function of the medium. They are, for example, much larger on 0.5 per cent agar than on a medium otherwise of the same composition but containing 3 per cent of agar. Furthermore, bacterial colonies are much smaller if they are abundant and packed closely together than if they are isolated. In all respects bacteriophage corpuscles exhibit exactly the same peculiarities as do bacterial colonies. Such a resemblance can only be due to the fact that the laws which determine the formation of the colony are the same whether they operate on bacteria or upon bacteriophage corpuscles.

For a long time it has been known that the principal factor limiting bacterial growth is the accumulation of products resulting from the metabolism of the bacteria. These products are harmful to the bacteria and prevent their development. It is possible to show that the products derived from bacteriophagy likewise exert a harmful influence on the bacteri-

ophage corpuscles and interfere with their development. From among many different experiments I select the most clear-cut. Doerr had noted that plaques do not form on a gelatin medium and he concluded that there is an antagonism between the gelatin colloid and the bacteriophage colloid. That this hypothesis of Doerr is erroneous can be demonstrated readily, for if a thin layer of gelatin is flowed over an agar surface the medium will prove to be suited to plaque formation just as is an agar surface. But the deeper the layer of gelatin the smaller are the plaques, and they no longer appear when the depth of the gelatin reaches about 3 millimeters. The inhibiting action of gelatin is not due, therefore, to the simple fact that it is of colloid nature. The single possible explanation for its inhibitory influence, as compared with agar, is that the latter is far more permeable than is gelatin to the products which form during bacteriophagy and which retard the action of the bacteriophage. When the gelatin layer is thin, diffusion of the products into the subjacent layer of agar more readily occurs and, consequently, the plaques are larger. Agar itself exerts exactly the same influence, for the lower the percentage of agar in the medium the more readily is the diffusion of these harmful products accomplished and the larger is the plaque. In brief, the phenomena which determine the formation of plaques are identical throughout with those

which regulate the formation of bacterial colonies.

It may be recalled, that I have stated that in order to obtain, in a clear-cut fashion, the results described, it is necessary to employ very active races of bacteriophage. Do not all bacteriophage races which may be isolated present these same characteristics? As a matter of fact, with a given bacterium different bacteriophage preparations may yield very different results. One may isolate a bacteriophage so powerful that a single corpuscle added to a culture of susceptible bacteria is sufficient to bring about a complete lysis in less than 12 hours—1 or 2 million corpuscles cause it in 2 to 3 hours. It is equally possible to isolate other races of bacteriophage at the other extreme, so weak that the addition of many thousand million corpuscles to a culture of the same bacterium does not lead to appreciable lysis. With such races it is necessary to apply very delicate and thorough methods in order to detect the presence of bacteriophage. Between these two extreme limits of activity there is every possible degree of potency.

What is the significance of these differences in activity? Experiment shows that the weaker the bacteriophage, the more slowly do the corpuscles reproduce at the expense of the bacteria and the number ultimately obtained is smaller. To make a direct comparison between the character of virulence in a pathogenic bacterium and the activity of a bacteriophage

race is simple. The virulence of a bacterium is the power to reproduce *in vivo*, and the degree of virulence is an expression of the rapidity of this reproduction. The same is true for the bacteriophage and its activity represents, indeed, a true virulence possessed by the corpuscles for the bacterium susceptible to their action. There is still another point of resemblance of great importance. We know that it is possible to enhance the virulence of a pathogenic bacterium, and that this can be done by making passages through susceptible animals. The same thing is true for the bacteriophage. By causing a weak bacteriophage to pass through a series of cultures of susceptible bacteria the virulence is enhanced. I observed this fact at the beginning of my studies and it has since been confirmed by all investigators, without exception.

But the resemblance between bacterium and bacteriophage does not stop here. We know that some of the pathogenic bacteria attack many species of animals, that is to say, they possess a virulence for different hosts, while other bacteria, on the contrary, are pathogenic for but a single species. It is the same for the bacteriophage. Certain races of bacteriophage manifest a specificity so strict that they attack but a single bacterial strain, even to the exclusion of all other strains of the same species. Other bacteriophage races attack all strains of a single species, and,

finally, still others attack all strains of several species, sometimes the strains being but very remotely related. For example, I have isolated bacteriophage races which were virulent for *B. coli,* the dysentery and paradysentery bacilli, and the cholera vibrio. Others isolated have been, at the same time, active for *B. coli* and *B. pestis,* or for *B. coli* and the staphylococcus. These facts have been confirmed by a number of investigators. Even experimentally, *in vitro,* it is possible to adapt a bacteriophage to the attack of bacteria belonging to a species for which it was totally inactive at the time of its isolation.

But, perhaps you may say, how can one know that it is the same bacteriophage corpuscle leading to bacteriophagy of the several bacterial species? Would not this same result be obtained if one were working with a fluid containing different bacteriophages? The following experiment satisfies this objection.

Let us take two races of bacteriophage, both capable of causing bacteriophagy of the same bacterium, but possessing characteristics of such a nature that the two races can be differentiated. Mix them and introduce a trace of the mixture into a culture of the susceptible bacterium. Then spread upon agar a loopful of this culture. After incubation plaques will appear. With a platinum wire touch the center of one plaque and transfer the wire to a fresh culture of susceptible bacteria. Repeat this operation with

several plaques. Study of the suspensions thus obtained will show that each of them contains a pure culture of bacteriophage of either one type or the other. It is thus easy to obtain pure cultures of a bacteriophage, and this can be readily understood since, as we have seen, each plaque is but a colony of bacteriophage corpuscles, the issue of a single corpuscle.

When one demonstrates that a bacteriophage derived from a single isolated plaque, and with even more certainty when one has repeated the isolation several times, possesses virulences for several bacterial species it is, indeed, certain that this polyvirulence is not due to a mixture of races but is an inherent part of a single bacteriophage corpuscle.

To summarize the facts disclosed by the experiments thus far described: we may say that the characteristics of bacteriophage corpuscles, their mode of reproduction, and their behavior approach very closely to those of pathogenic bacteria. Pathogenic bacteria invade the body of the superior animal and, by virtue of their virulence, grow there, causing a disease which results either in the death of the organism invaded or in recovery. When the latter occurs it is usually followed by an immunity. Bacteriophage corpuscles invade bacteria, and, thanks to their virulence, thus cause a fatal disease. This is bacteriophagy. But we will see in what is to follow

that bacteriophagy is not always fatal, that bacteria may recover, and that in such cases an immunity to the bacteriophage results.

CHAPTER II

Bacterial Mutations

THE phenomenon of bacteriophagy as it has just been described may appear relatively simple and, indeed, it would be if the bacterium were an exception among living beings, if it lacked the faculty of reacting or adapting itself to transitory, harmful conditions. It is, however, necessary to reflect for but a moment to appreciate that without this faculty life would be impossible. Undergo adaptation or die, such is the dilemma confronting all living beings.

If bacteria inevitably succumbed to the action of bacteriophage, before long bacteria would cease to exist, and this in turn would entail the disappearance of bacteriophage, which, lacking a host, could not survive. One cannot affirm that certain bacterial species have not met with such a fate in the past. It would only be necessary for a bacteriophage to exalt its virulence to such a point that whatever might be the environmental conditions none of the bacteria of a species would be able to react effectively and thus acquire an immunity to the aggressor. Such a species would disappear. That man may some day possess

the means of experimentally accomplishing such an enhancement of virulence in a bacteriophage is not impossible. Ideal hygiene would thus be realized. But we have not yet reached that point.

Returning to facts as they exist, we find that bacteriophage is profoundly influenced by the conditions of its environment. The principal conditions which favor it are a temperature which does not rise above 40° C., and a slight alkalinity of the medium, a pH of from 7.2 to 8.2. It is also essential that the bacteria contained in the medium be not too numerous. As we have seen, the substances liberated, or which are formed, in the course of the phenomenon of dissolution interfere with the action of the bacteriophage. Complete dissolution takes place only when the bacteria do not exceed a certain number, a number which varies in accordance with the bacterial species involved, for example, 250 million per cubic centimeter for the cholera vibrio; 1,000 million for the staphylococcus or for *B. dysenteriae.* That this number should vary according to the species is obvious, for the products which result from the dissolution of one bacterium, the cholera vibrio, for example, may well be more harmful for the bacteriophage than those originating during the dissolution of the dysentery bacillus. When the number of bacteria exceed a certain limit harmful products accumulate, the phenomenon becomes retarded and then finally stops

before all of the bacteria have succumbed. We will find, also, that optimal conditions vary with different races of the bacteriophage for, like the bacterium, the bacteriophage may adapt itself to unfavorable conditions. Thus it is, that one may experimentally adapt a bacteriophage to cause complete lysis in definitely acid media, a fact which explains why certain races, even at the time of isolation, are able to provoke a complete dissolution in such media.

Let us combine a bacteriophage which has not undergone adaptation with a susceptible bacterial culture under conditions which are unfavorable for bacteriophagy. The process begins in a normal fashion, the medium becoming more or less clear, the degree depending upon how closely conditions approach the optimum. But soon the medium again becomes clouded, for some of the bacteria have resisted and have begun to develop again. If we restore favorable conditions bacteriophagy will take place normally and the medium will become perfectly clear. Thus it will remain for a period, which may vary from a few hours to a few weeks, when a definite bacterial growth makes its appearance and the medium becomes turbid. These cultures which develop after the action of bacteriophage are termed "secondary cultures."

Secondary cultures can be obtained just as effectively by the use of a bacteriophage of low virulence

as with a race of high virulence acting under unfavorable conditions. However favorable may be the conditions, with such a race of bacteriophage secondary cultures will uniformly develop. This should occasion no surprise, for do not animals appear to resist bacterial invasion better when the virulence of the attacking parasite is low? When the virulence of the bacteriophage is very low no visible macroscopic lysis occurs and only the abnormal appearance of the cultures developing on agar discloses its presence. With such races it is necessary to enhance the virulence by several serial passages in order to have the action manifest in a fluid medium. Indeed, as I have shown and as Flu has since confirmed, in certain cases the bacteriophage may disappear in the first passage, being destroyed by the bacteria. But, if the virulence of the bacteriophage approximates the maximum degree dissolution will be normal, the medium will clear completely and will remain clear for a longer or shorter time, when a definite secondary growth will appear.

In brief, the development of bacteriophage-resistant bacteria, with the appearance of secondary cultures, is determined by the degree of virulence of the bacteriophage and by the closeness with which the environmental conditions approach the optimum for bacteriophagic action. The lower the virulence of the bacteriophage or the less satisfactory the condi-

tions for the bacteriophage, the greater will be the tendency for secondary cultures to develop.

Here it may not be out of place to call attention to a situation which has led many bacteriologists into error. When a weak bacteriophage acts upon a culture of susceptible bacteria or, indeed, when a potent bacteriophage acts under very unfavorable conditions evidence of dissolution may not be apparent and yet an increase in the number of bacteriophage corpuscles may occur. The authors to whom I refer have concluded that a regeneration of bacteriophage can take place without a destruction of bacteria. This interpretation indicates a failure to analyze what takes place. We have seen, and experiments are conclusive in this respect, that the virulence of a bacteriophage bears a direct relation to the rapidity and the intensity of its multiplication. A bacteriophage of low virulence reproduces slowly and the number of bacteria which divide is infinitely greater than the number of bacteria which are dissolved and, consequently, macroscopically at least, nothing indicates that the phenomenon of bacteriophagy is taking place. Because of the diminished rate at which the phenomenon progresses a large number of bacteria acquire resistance before any visible clearing of the medium can take place and these bacteria thenceforth multiply normally, for they have become refractory to the action of the bacteriophage. It is, indeed, most illogi-

cal to conclude that dissolution does not take place in the presence of a weak bacteriophage or with a strong bacteriophage acting under conditions so unfavorable as to retard multiplication. When one observes all the intermediaries between a rapid, complete dissolution and no visible dissolution, how may one conclude that in the latter case some destruction does not occur? Is it not logical to assume that under such conditions the number of bacteria undergoing dissolution is relatively small in comparison with the number developing through the continued multiplication of those organisms which survive?

In all secondary cultures resistant bacteria and bacteriophage corpuscles co-exist. In such cultures it is easy to destroy the bacteria without eliminating bacteriophage. It is only necessary to raise the temperature to between 56 and 60° C. for, although the bacteria are killed at this temperature, the bacteriophage corpuscles are not destroyed until a temperature of 74 to 75° C. is reached. The opposite procedure is not yet possible, for as yet we have no method—physical, chemical, or biological—of general application, which will eliminate bacteriophage and leave the bacteria intact and viable. To none of the commonly used antiseptics are the bacteriophage corpuscles more susceptible than are the bacteria. In specific isolated cases, as Schultz and Kroger have recently shown, methylene blue is effective, causing the death, or in-

activation, of certain races of bacteriophage without impairing bacterial viability. But this sensitivity to methylene blue is not general, for other races of bacteriophage are not modified by it in any demonstrable way. It is unnecessary to point out that it would be of extreme importance to find a generally applicable method which would uniformly kill the bacteriophage and exert no harmful effect upon bacteria. Applied to a study of those symbioses between bacteria and bacteriophage as they frequently occur in nature, as we will see later, such a method of separation would be of the utmost value. The method of separation heretofore used, that of plating out, is not always effective. While it may serve to separate the two beings when the symbiosis has been but recently established, it is no longer serviceable when the symbiotic state has persisted throughout several generations.

Let us implant upon agar, in such a way as to obtain isolated colonies, a quickly developing secondary culture, that is to say, one which has developed before the media has cleared and which has been obtained by using a bacteriophage of low virulence. After incubation let us remove a number of colonies and reinoculate them into tubes of bouillon. After a further period of incubation we will have several cultures, each one the issue of an isolated colony. If we subject these cultures to the action of the bacteriophage which served to produce the secondary cul-

ture from which these bacteria were derived, we will find that bacteriophagy takes place in some of these tubes, while others remain turbid. When we filter these turbid tubes we will find that some of the filtrates will contain bacteriophage while others will not.

From such experiments it appears that the colonies which have been derived from the agar represent three different types—First, colonies of normal bacteria free of bacteriophage corpuscles and susceptible to the action of bacteriophage; second, colonies of bacteria free of bacteriophage corpuscles, that is, colonies which are ultrapure but abnormal in that they resist the action of bacteriophage; and third, mixed colonies, those in which bacteria and bacteriophage corpuscles co-exist. The bacteria of these colonies are always resistant to the bacteriophage.

The relative number of colonies of these three types is extremely variable, depending upon the conditions of the experiment. Thus it was that after some experiments made early in my studies I found only colonies of types 1 and 2. Bordet next revealed colonies of type 3, and Kuttner demonstrated cultures composed of types 1 and 3. Finally Bordet, working with *B. coli,* encountered a mixture of the three types.

If we subject cultures of the three types to subculturing a great many times, we will find that each type possesses its own peculiar attributes. Cultures of the

first type are ultrapure and susceptible, and they remain normal indefinitely. The bacteria of these cultures, observed under the microscope, are morphologically identical with those of normal cultures typical of the species. An examination of their characteristics will show that in all respects they conform to the type species, and since they remain so in successive subcultures we may conclude that these bacteria are absolutely normal, undergoing no mutation. And as we will see by experiments to be described shortly, we can also conclude that these bacteria have not been affected by the bacteriophage, a fact which, as may be said in passing, is only compatible with the corpuscular nature of bacteriophage.

In the course of successive transplantations those bacteria yielding cultures of type 2, resistant and ultrapure, return gradually to the normal type. After about 20 transplants they have become as susceptible to bacteriophage as are normal bacteria of the same species. This shows us that the resistance acquired by the bacterium is comparable throughout to an acquired immunity and that gradually it is lost in the course of successive generations. This resistance is, therefore, an immunity acquired to a foreign principle and through the action of this principle, since the immunity diminishes gradually when the inciting principle is lacking. Furthermore, if in the course of the transfers upon agar we test the behavior

of isolated colonies, we will find that resistance to the bacteriophage is transmitted unequally to the descendants of the originally resistant strain. In the first subculture we will find some colonies formed of resistant bacteria and others made up of bacteria as susceptible as are normal bacteria. After about 20 subcultures resistant bacteria are no longer to be found.

Microscopic examination of these diverse colonies frequently shows that resistance to the bacteriophage is accompanied by morphological changes. Very early in my studies I noted this correlation of morphology and resistance, for I observed that dysentery bacilli became coccoid in form only to return to their normal form when, through successive generations, the resistance was lost.

Another, and perhaps more interesting, fact bearing on the nature of these organisms is that with certain bacterial species, including dysentery bacilli, with which I first observed the phenomenon, a resistance to bacteriophagy is accompanied by a loss of agglutinability by specific sera. From this it would seem that the acquisition of resistance must produce a change in the surface tension leading to a modification in the electrical charge borne by the bacterium. Undoubtedly it would be more correct to say that resistance is due to the modification of the charge. But whatever the sequence of events, loss of agglutinabil-

ity is essentially associated with resistance to the bacteriophage, for it persists only for the duration of the resistance. Whenever in the course of subculturing, the bacteria again become susceptible they likewise again become normally agglutinable.

Through the influence of bacteriophage there occurs, then, transitory modifications in the characteristics of bacteria, which revert to the normal type when the bacteriophage, the cause of the mutation, is no longer present. Bacteriologists have said that we cannot call "mutation" a modification which is reversible. This is not correct, for the geneticist employs the terms "reversible" and "irreversible" mutation.

Passing to a consideration of colonies of the third type, that is, the mixed colonies in which resistant bacteria and bacteriophage corpuscles are combined, we enter upon an extremely complicated situation and one which, up to the present time, is but inadequately explained.

In the first place let us note that if serial cultures of such colonies are made in liquid media one always obtains, even after an indeterminate number of transfers, mixed colonies composed of resistant bacteria and bacteriophage corpuscles. If, in the course of these successive transplants in liquid media, transfers to agar are made it will be found that in the earliest platings colonies of all three types—ultrapure re-

sistant, ultrapure susceptible, and mixed resistant—are obtained. As the successive transfers are made the colonies of the first two types become less and less abundant, until finally all of the colonies are mixed. This shows that a symbiotic relationship between the bacterium and the bacteriophage corpuscle becomes established. Although at first a separation of the two constituents is possible, later it becomes more difficult and finally it is impossible. When a symbiosis has become permanent it even becomes difficult to recognize that such a symbiosis exists, for through a process of adaptation, the bacteriophage, developing always at the expense of the same bacterium, gradually loses the faculty of developing at the expense of other bacterial strains of the same species. The symbiosis becomes obligatory. This is, in effect, the formation of what might be termed a "microlichen." Examples of such a perfect symbiosis between host and parasite are, however, frequent in nature. It is only necessary to mention the case of cattle and the protozoan, *Piroplasma bigeminum,* the cause of Texas fever. This parasite is present in the blood of all cattle in hot countries. It reproduces with extreme slowness and in no way disturbs the health of these animals. It is, however, a dangerous parasite for if cattle are brought in from a district in which Texas fever is not present they quickly contract the disease and it is almost always fatal within

a short time. A hundred examples of such a symbiosis may be found in the classic treatise of Buchner.

We have seen that a bacterium-bacteriophage symbiosis occurs when serial transfers of a mixed colony are made. It also develops without transfers when a mixed colony is simply permitted to undergo aging, as is evident from the fact that if isolations upon agar are made from time to time we will find that the number of ultrapure colonies, susceptible or resistant, progressively diminish and finally only mixed colonies constituted of resistant bacteria and bacteriophage corpuscles remain.

It may be mentioned that a number of authors have failed to distinguish between ultrapure resistant colonies and contaminated, mixed, resistant colonies. This has served not a little to render more confused a question which, although highly complicated in its consequences, is in reality very simple in principle.

We have said that in the first platings of a secondary culture the three types of colony are to be found in very variable proportions. The distribution of the types will depend upon the conditions surrounding the formation of this secondary culture, conditions such as the degree of virulence of the bacteriophage, the number of bacteria present, the reaction of the medium, the temperature, etc. But whether a secondary culture is permitted to age, or whether serial trans-

plants are made, ultimately the susceptible and the resistant ultrapure types will always disappear and there will remain only the bacterium-bacteriophage symbiosis which is permanent and in which the bacteria are always resistant.

But, perhaps you may say, as soon as all of the bacteria are resistant how can the bacteriophage corpuscles multiply? Since these corpuscles continue to be present throughout the course of successive transplants and since the bacteriophage is an obligate parasite it must be that they multiply at the expense of resistant bacteria. This is true. But the fact that a bacterium resists destruction does not imply that bacteriophage corpuscles cannot develop at its expense. The resistant bacterium contracts what might be termed a chronic disease. For that matter, all of the true symbioses are chronic diseases, in which the virulence of one of the components is balanced by the resistance of the other. Nature presents many situations of this kind, indeed, it is only because parasites have retained the power to reproduce at the expense of a host which has acquired a resistance, that the innumerable symbioses which have developed throughout the course of the ages are possible.

No one will deny, for example, that the symbiosis of an alga and a fungus, known under the botanical term of lichen, is not a perfect symbiosis. It is so perfect that for a long time it was believed that the

lichen was a botanical unit. In this symbiosis the alga is the host and the fungus is the parasite, as is demonstrated by the fact that sometimes one sees the virulence of the fungus become enhanced and the alga totally destroyed. The symbiosis is then broken to the advantage of the fungus, but the advantage is of questionable value, for the host, being dead, does not permit further reproduction. But usually the symbiosis persists indefinitely and the fungus continues to reproduce at the expense of the alga. Another example is provided by the Myxobacteriaceae, so well studied by Taxter and by Pinoy, which are formed by Myxomyces developing solely at the expense of the bacterium. But this does not prevent the symbiosis from continuing indefinitely. Ultimately, a state of equilibrium between the resistance of the host and the virulence of the parasite is established. It is needless to multiply examples, for those which have been cited are comparable in all respects to the symbiosis established between the bacterium and the bacteriophage.

Bacterium-bacteriophage symbioses are extremely frequent in nature. Indeed, it could not be otherwise, for the bacteriophage is a normal inhabitant of the intestinal tract of all animals, where it forms natural "secondary cultures" with the usual bacteria of the intestinal flora, with *B. coli* in particular.

Bacteriophage corpuscles are widely disseminated throughout the external environment with the excreta.

They may be found in the water of rivers, in wells, in the soil, on dust particles floating in the air, and, in brief, in all things susceptible to contamination by dejecta. In all of these places the bacteriophage corpuscles are found in connection with bacteria. Colony isolations made from a natural secondary culture, for example, from a fragment of normal or pathological stool, inevitably yield the three types of colony which are found in experimental secondary cultures. If we select by chance a colony for cultivation we may obtain an ultrapure colony, either susceptible or resistant, or a mixed colony. In the first case this ultrapure colony will be the origin of an ultrapure, normal bacterial strain presenting fixed characters. In the second case it will yield a mixed culture in which, throughout the course of subcultures, resistant bacteria and bacteriophage corpuscles will persist, affording thus an abnormal *mutating* bacterial strain. One can readily understand that among stock cultures there may be—and, indeed, they have actually been demonstrated—both ultrapure bacterial strains and strains of the same species contaminated by bacteriophage corpuscles.

Is a bacterium which has an acquired resistance to a given bacteriophage resistant to all other races? This question has been widely discussed simply because many authors have applied an inadequate method to its solution. I have always affirmed that in con-

ducting an experiment dealing with the bacteriophage it is not sufficient to use but a single bacterial strain and then draw conclusions from the result. Such procedure invariably leads to error. Although there are some serious investigators who are aware of the difficulty, it must be admitted that there are others who fail to recognize the danger and continue to draw, from a single experiment, conclusions which they deem final. This erroneous method is particularly obvious in connection with the question of resistance. If we work with the cholera vibrio we will find that the resistance acquired to a bacteriophage extends to all other bacteriophage races virulent for this bacterium, and the resistance acquired is proportionate to the virulence of the bacteriophage. A resistance acquired against a bacteriophage of low virulence may be overcome by a bacteriophage possessing a higher virulence. If, on the contrary, we work with *B. coli* we will find that the resistance acquired to one bacteriophage usually does not extend to another. Between these two extremes may be found the diverse manifestations of resistance exhibited by other species of bacteria. The reason for these differences in bacterial behavior must be found in the phenomenon of adaptation. *B. coli* is a bacterium which, in its natural habitat, is continually in a state of resistance against varied races of bacteriophage, and this fact must influence its behavior toward the latter. This is

the more probable since it appears that the more closely a bacterial species resembles *B. coli* with respect to its relationship to bacteriophage the greater does its resistance resemble that of *B. coli.*

Many authors, Bronfenbrenner for example, have thought that the behavior of *B. coli* was hardly compatible with the theory which identifies resistance to the action of bacteriophage with phenomena of immunity. As a matter of fact the opposite is true, for comparable facts are frequent in nature, as a few examples will clearly show. A cow which has acquired an immunity to one strain of the virus of aphthous fever may remain susceptible to another strain as Vallée has very well shown. Indeed, this behavior is not limited to filterable viruses. We know that there is no general immunity to the pneumococcus but that this immunity is limited to certain strains, and that in the case of an acquired immunity to a Type IV pneumococcus the resistance is limited to this single strain. Even more significant is the type of immunity acquired by an animal for *B. coli,* for each strain of this bacterium seems to behave like a bacterium of a distinct species. Here the resemblance is as close as it can well be, for, as in its behavior toward bacteriophage, each strain of *B. coli* acts in all respects like a distinct species.

Let us consider another error of interpretation. Resistance to the bacteriophage is accompanied, as we

have seen, by changed characteristics, giving new attributes to the bacteria which resist. But Bronfenbrenner, for example, finds that "it is difficult to perceive how such changes could occur as a direct result of the development of active immunity by bacteria". Again let us state that nature is replete with examples showing that the characters of living beings are modified by the action of a parasite. Indeed, examples are so common that one might formulate a law to the effect that resistance to the action of a parasite is accompanied by a modification in the characteristics of the host and that the new characters are often transmissible to the descendants. But one example from among thousands need be cited. Noël Bernard has shown that tuberization, that is, the formation of tubers by a plant, is the specific result of the resistance of the plant to the action of the parasitic fungus. The change is transmitted to the descendants even in the absence of the fungus, but where the parasite is lacking it tends to regress in the course of successive generations. The potato offers an example of this. Frequently, in Europe, this regression has gone to such a point that they have been forced to stop the cultivation of certain varieties which gave smaller and smaller tubers. In such cases, to re-establish tuber formation, it is only necessary, as Noël Bernard has shown, to contaminate the soil with cultures of the specific fungus always found on the tubers of the

wild potato of South America, where the symbiosis is permanent. Only this single example need be cited, for the situation is entirely comparable with the phenomenon of mutation as seen in bacteria modified through the influence of bacteriophage.

In our consideration of ultrapure, resistant colonies we have seen that under the transitory action of bacteriophage bacterial transformations correlated with an acquired resistance occur. We have seen further that these modifications are not transmitted to the descendants uniformly and that successive cultures derived from the resistant, ultrapure colonies reveal intermediaries between the coccus form and the normal bacillary form, between absolute inagglutinability and normal agglutinability. There is induced, then, by the bacteriophage a "microbic dissociation" according to the terminology of Hadley, but we will see that such a term is incorrect and decidedly misleading, for this so-called "dissociation" is simply a modification of characters induced by the bacteriophage. Definite proof that this is the case is afforded by the facts that these modifications are proportionate to the resistance and that they become diminished and then finally disappear as resistance fades. These mutations, in this case, reversible, are, then, abnormal phenomena resulting from an abnormal state, that is from a pathological state of the bacterium. They are the visible evidences of a reaction on the part of the bac-

terium to a pathogenic agent which tends to destroy it.

In the case of ultrapure, resistant bacterial strains the mutations are temporary. What happens in the mixed strains, derived experimentally or occurring under natural conditions, where resistant bacteria and bacteriophage corpuscles co-exist? In this case, does the cause of the mutations undergone by the bacteria continue to exercise its action indefinitely and, if so, what characters do these mutations assume?

Early in my studies I conducted experiments dealing with the changes in the fermentative powers of dysentery bacilli isolated from secondary cultures which had undergone a number of transfers. I was never able to arrive at any definite conclusion for although changes in activity could be produced, they lacked uniformity. In one experiment certain sugars were fermented, in another they were not, or entirely different sugars were broken down. In continuing the cultures by means of isolated colonies I demonstrated that the different colonies did not behave in the same fashion. Because of this lack of uniformity in behavior I feel justified in saying that in spite of the work done by many investigators the question of the mutations induced in bacteria through the action of bacteriophage still remains quite obscure. Facts have been accumulated, often apparently contradictory, and for this discrepancy we have seen the reason. As a

rule each investigator has worked with but a single bacterial species and has utilized but a single race of bacteriophage in his studies. As a matter of fact it is still impossible to formulate laws, that is, to predict what will take place in any particular case, for mutations occur in all directions, involving different characters. They are transitory or permanent, one transformation or another will take place with a single bacterial strain. All that may be said is that through the influence of bacteriophage the bacteria react and this reaction is accompanied by changes in its characteristics, changes which occur in an apparently irregular fashion. Obviously this disorder is apparent only, and appears such solely because we do not know the nature of the intimate mechanism of resistance.

In the present state of our knowledge it must suffice simply to review the different variations which have been observed up to the present time. These variations involve motility, morphology, capsule formation, chromogenesis, proteolytic power, fermentative power, agglutinability, virulence, and the formation of filter-passing forms. At the moment we will not consider variations of the last two types.

We have already seen that bacilli assume a more or less perfect coccus form at the time when they acquire resistance, and the return to the typical bacillary form parallels the loss of resistance. From old secondary cultures or from fresh cultures, obtained after re-

peated transfers of young secondary cultures of dysentery, paradysentery, typhoid, and paratyphoid bacilli, *B. coli,* and the cholera vibrio, I have isolated stable coccus forms. These remain indefinitely (from 6 months to 3 years) cultivable in the new form. This extreme in morphological mutation is, for all of the species mentioned, an organism whose cultural and morphological characteristics are identical with those of the enterococcus. I have obtained the same extreme mutation starting with mixed cultures of the staphylococcus. One may well ask if the enterococcus is, indeed, a botanical species, or if it is not a form resulting from mutations common to several bacterial species. Starting with secondary cultures of staphylococci I have likewise obtained chained forms which retained this character through successive cultures.

A loss of motility is readily observed in secondary cultures of motile bacteria, and with *B. coli* in particular, non-motile, stable races can be obtained. From a secondary culture, several weeks old, of *B. typhosus* on agar, I have isolated a colony which was non-motile and which in its lack of fermentative power resembled *B. alkaligenes.* A non-motile stable mutation of the cholera vibrio can only be obtained after a great many transfers in mixed cultures.

I have observed that bacteria (*B. dysenteriae, typhosus, coli,* the staphylococcus, and the cholera vi-

brio) may secrete a mucoid substance when acted upon by the bacteriophage. Bordet and Ciuca have recorded the permanent transformation of *B. coli* into a form presenting all of the characteristics of the Friedländer bacillus.

In so far as chromogenesis is concerned I have obtained permanent mutations of both *Staphylococcus aureus* and *citreus* into *Staphylococcus albus.* Feigin has observed the formation of pigmented races of proteus, and I have noted the same thing in the case of the cholera vibrio.

It is very easy to obtain non-proteolytic races of the staphylococcus; in fact this seems to be a common characteristic of all resistant staphylococci.

Variability in fermentative characters is one of the most frequent phenomena and may be observed with all bacteria resistant to the bacteriophage, but, as I have already noted, it is impossible to establish any rule governing the direction which the variations will take. In sa far as the cholera vibrio is concerned a complete loss of fermentative power seems to be the extreme change and it is without doubt the same for the typhoid bacillus.

We have already spoken of the loss of agglutinability. One may readily isolate, from old secondary cultures, races which remain inagglutinable in successive cultures. Of all the species with which I have worked the cholera vibrio loses its susceptibility to

agglutination most slowly. The successive mutations induced in bacteria by bacteriophage can best be illustrated by describing some of the observations made upon the cholera vibrio during 1927, in India.

First let us consider experimental mutations. This experiment was performed with a vibrio isolated from the stool of a cholera patient on the first day of the disease. This vibrio was then maintained under artificial cultivation during the period of a year, and its characters have remained throughout those of a typical cholera vibrio. Obviously it is a strain which does not undergo spontaneous mutation. Through the action of a potent bacteriophage a culture of this vibrio was dissolved perfectly within 3 hours at 37° C., but a secondary culture appeared after 5 days. When 0.1 cc. of this secondary culture was inoculated into a tube of peptone water the medium remained sterile, but when 0.1 cc. of this same secondary culture was spread over a Petri dish there developed, after incubation, about 20 small colonies, varying in size from those approaching the limit of visibility to those having a diameter of about 2 mm. Six of these colonies were cultured and studied, and the characteristics of cultures derived from each are listed in the following table. Table I.

For purposes of study several tubes of bacteriophage were prepared, that is, tubes containing cultures of this vibrio dissolved through the action of a

Table I

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentations							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
1	slight	−	−	−	+	+	−	−	+	+	+	+	Bacilli and vibrios
2	+	+	−	1:50	−	+	−	−	+	+	+	+	Bacilli and vibrios
3	slight	−	−	1:50	−	+	−	−	+	+	+	+	Bacilli and vibrios
4	+	+	+	1:1000	+	+	−	−	+	+	+	+	Vibrios, cocci, and bacilli
5	+	+	−	1:250	−	+	−	−	+	+	+	+	Bacilli and cocci
6	+	+	−	1:1000	−	+	−	−	+	+	+	+	Bacilli and cocci

powerful bacteriophage. These were filtered through a candle. The strains of the vibrio were maintained in culture, and as repeated subculture has proved, the characters remained fixed. These vibrio strains were, therefore, not spontaneously mutating. But in the course of our studies some of the filtrates developed a turbidity after variable lengths of time. One of them showed a slight clouding 17 days after having been sealed, which excluded the possibility of the clouding being due to a contamination. In other tubes the clouding appeared six weeks after the tubes had been sealed. The characters of the organisms thus developing are certainly not those of a contaminating bacterium. Such secondary cultures represent, undoubtedly, the passage through the filter of "protobacterial" filterable forms and these "protovibrios" then develop into bacterial forms. Thus, when we inoculate 0.1 cc. into a tube of peptone water there is no growth even after an incubation period of 15 days. When 0.1 cc. is spread upon the agar a few small colonies will appear, but only after incubation for 4 days. These colonies will vary in size from the limit of visibility to the naked eye to colonies a fraction of a millimeter in diameter. Six of these colonies were studied, with the results here indicated. Table II.

It may be mentioned that in the course of other studies of similar cultures we have found, upon three occasions, cocci developing a yellowish pigmentation.

TABLE II

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentations						Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
7	slight	−	−	−	−	−	−	−	−	−	−	−	Bacilli
8	−	+	−	−	+	+	−	−	−	−	−	−	Small vibrios
9	−	−	−	−	−	−	−	−	−	−	−	−	Cocci and short vibrios
10	−	+	−	−	+	+	−	−	−	+	+	−	Bacilli
11	−	−	−	−	−	−	−	−	−	−	−	−	Vibrios and cocci
12	−	−	−	−	+	−	−	−	−	−	−	−	Cocci

Let us now turn to mutations occurring within the body. In all of the cases of cholera studied we have prepared a filtrate of the stool upon each day of the disease. These filtrates, distributed in tubes, were immediately sealed for purposes of possible future study. Several of these filtrates have developed a turbidity, due, as microscopic examination has shown, to bacterial growth. But all of the tubes behaving in this fashion were derived from the stools of patients in whom we had demonstrated the simultaneous presence of the vibrio and the bacteriophage. We have studied many of these secondary cultures, and the results obtained with one of them are here presented. When 0.1 cc. was inoculated into a tube of peptone water it remained sterile even after incubation at 37° C. for 8 days, but when 0.1 cc. was spread upon agar there developed after 4 days a few very small colonies, as in the preceding case. Study of six of these colonies gave the results presented in Table III.

And, finally, for purposes of comparison, here are the characters of 8 strains of the vibrio isolated from chronic carriers by Colonel Stewart, Professor of Hygiene at the School of Tropical Medicine at Calcutta. These vibrios (Table IV) had undergone many subcultures in the laboratory. It should be stated that No. 22 at first, at the time of isolation, had the vibrio form.

It is easy to understand that the extreme forms

Table III

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentations							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
13	−	−	−	−	−	−	−	−	−	−	−	−	Cocci and short vibrios
14	−	−	−	−	−	−	−	−	−	−	−	−	Cocci and bacilli
15	−	+	−	−	−	−	−	−	−	−	−	−	Cocci and bacilli
16	−	−	−	−	−	−	−	−	−	−	−	−	Cocci and short vibrios
17	−	−	−	−	−	+	−	−	−	+	+	−	Bacilli
18	−	−	−	−	−	−	−	−	−	−	−	−	Bacilli and small vibrio

found under experimental conditions will not be isolated from the chronic carrier, for one will pick up on the isolation plates only colonies composed throughout, or in part, of vibrio forms. But we have sought and always found colonies formed of bacilli and cocci, analogous to those which have been isolated from secondary cultures, when we have studied the isolation plates inoculated with stools of carriers.

As may be seen by a comparison of these tables, the mutations undergone by the vibrios within the intestinal tract of chronic carriers (in whom we have always been able to demonstrate a bacteriophage virulent for the cholera vibrio) are of the same order as those produced experimentally through the action of the bacteriophage.

It is quite obvious that the successive mutations take place through the loss of characters, but each character varies independently of the others. Let us take the character of "vibrio-form" for example. In the case of colony 24 vibrio forms were still to be found even though all of the other characters had been lost, while in colony 6 the vibrio form was no longer found, although the other characters of the cholera vibrio, with the exception of sensitivity to the bacteriophage, were retained. Even agglutinability to the titer of the serum persisted.

As for the nitroso-indol reaction, which indicates the simultaneous secretion by the organism both of

TABLE IV

Number	Motility	Nitroso-Indol Reaction	Hemolysin Production	Agglutinability	Susceptibility to Bacteriophage	Sugar Fermentations							Morphology
						Dextrose	Lactose	Dulcite	Mannite	Saccharose	Maltose	Salicin	
19	+	+	+	+	+	+	+	+	+	+	+	+	Long vibrios and cocci
20	+	+	+	+	+	+	+	+	+	+	+	+	Short vibrios and bacilli
21	+	+	+	+	+	+	+	+	+	+	+	+	Short vibrios and bacilli
22	+	+	+	+	+	+	+	+	+	+	+	+	Bacilli
23	+	+	+	+	+	+	+	+	+	+	+	+	Short vibrios and bacilli
24	slight	+	+	+	+	+	+	+	+	+	+	+	Short vibrios and bacilli
25	+	+	+	+	+	+	+	+	+	+	+	+	Long vibrios
26	+	+	+	+	+	+	+	+	+	+	+	+	Long vibrios and bacilli

indol and of a reductase transforming nitrates into nitrites, this may be lost in organisms which retain all of the other characters, (see colony 3) and may be conserved in others which have lost almost all of their other distinctive attributes (see colony 13).

In general, it may be said that the character of agglutinability is the one most readily lost, although observations made upon patients and during the course of epidemics show that the character of virulence is still more fragile. Next in lack of stability is the character of secretion of hemolysin for the red blood cells of man. In so far as fermentative characters are concerned, the most stable is the fermentation of dextrose. *But each character is an entity which is lost or retained in an independent fashion.* This observation indicates that these transformations represent *mutations* in the true sense of the word, for in all of the mutations studied up to the present time, this is precisely what has been observed, namely, characters undergo modification in an independent manner.

Various authors, Hadley among others, have sought to explain the transformations undergone by bacteria in terms of a "life cycle". Such an explanation cannot be correct, for a "life cycle", such as is known with the protozoan, consists of an orderly sequence of definite transformations. The term "bacterial dissociation" gives a false conception in that it implies a spontaneity of the phenomena, such as would result

from the existence of a life cycle. This is not shown by experiment. There are two phenomena in nature leading to the transformation of living beings; the life cycle and mutation. In the first phenomenon the transformations are orderly; in the second they occur in a disorderly way. When we observe that beings undergo transformation, if we would determine whether they are expressions of a life cycle or evidences of mutation, we must show whether they occur in a regular or in an irregular manner. Bacterial transformations are certainly the most disorderly ever observed. They result from mutation. The transformations undergone by bacteria are, as the experiments described demonstrate, bacterial mutations and a bacterial strain in which these transformations take place without an *apparent* cause is a mutating strain. The many studies which I have made during the last 12 years have convinced me that such bacterial mutations are produced exclusively through the action of bacteriophage. It is, however, obvious that there must be bacterial diseases other than bacteriophagy and that these may also be the cause of mutation. But in any case, a mutating bacterial strain is never a normal strain. It is a strain affected by a chronic disease due to the presence within it of some foreign parasite. Perhaps it is desirable to mention that I do not include as mutations those transitory transformations in morphology which take place as the result of a modifica-

tion of the medium; through the action, for example, of certain antiseptic substances. Here the phenomenon is of an entirely different nature.

In view of everything thus far disclosed with reference to the mechanism of bacterial mutation I must retain my belief that all who have observed mutating strains have dealt with mixed bacterial strains representing a bacterium-bacteriophage symbiosis, and such strains are extremely common in nature. This conviction is based upon the following facts. The descriptions of the cultural characteristics of the strain, as recorded by the various authors, correspond completely to that of an experimentally produced mixed strain. According to these authors mutability is a native property of certain strains only. Obviously this could not be the case if they resulted from a cyclogeny, for then mutability should be a general fact and not peculiar to certain strains. All strains placed under identical conditions should undergo mutation. Finally, if the mutations resulted from a cyclogeny it should always operate in the same direction for a given bacterium, otherwise each bacterium must possess an infinite number of different cycles. This is, indeed, rather difficult to assume.

In reality, the fact that we are able at will to transform a fixed strain into a mutating strain, identical with naturally occurring mutating strains, shows clearly that in all cases the cause of the mutation is

the same and that these strains undergoing mutation under natural conditions are strains in which bacteria and bacteriophage exist in symbiosis.

As for the view of Hadley concerning the behavior of bacteriophage "and of its mythical, mutation provoking powers" I regret to have to say that this author seems unaware of the basic biological phenomena of the general influence of symbiosis as a cause of mutation.

Among the innumerable known instances of symbiosis, it is impossible to find a single one which does not profoundly modify the characters of the host. Bearing in mind all known biological facts I believe that an animal or vegetable species is absolutely fixed only so long as a foreign agent, internal or external, does not intervene. Each change in attributes is the result of an adaptation. The adaptation, resulting in a change in the characters, takes place slowly if the operating cause is external, that is, if it is some change in the environmental conditions. Such a modification is regressive, for if the living being is restored to the initial environmental conditions a return to the primitive characteristics results. Adaptation takes place suddenly under the action of a foreign *internal* cause and this foreign internal cause can be nothing other than a parasite. In this case the mutation may be permanent, remaining even after the cause has disappeared. The causes of mutation, that is, of sudden

changes in character, are parasitism and symbiosis. And the latter, although simply a particular type of parasitism, is especially important.

In so far as bacteria are concerned, modifications of character are always the result of a process of adaptation. These modifications of character are generally slight and are always reversible when they result from an adaptation to environmental conditions, but they are profound when they reflect an adaptation to new internal conditions, created by the presence of invading parasitic corpuscles. In the latter case it seems that the modification may be permanent even though the cause of the change disappears, that cause being destroyed by the bacteria or expelled during the process of the transformation of the bacterium into its filter-passing form. The most interesting situation is encountered when the bacterium and the bacteriophage are united in a symbiosis. Then the cause of the mutation persists indefinitely, resulting in a mutant bacterial strain.

I well appreciate the fact that many of these statements will hardly be accepted by many bacteriologists who still retain a belief in the fixity of the bacterial species, but might I suggest that these experiments, very readily performed, be repeated? It is indeed strange that more bacteriologists have not had sufficient curiosity to do so already.

CHAPTER III

The Nature of Bacteriophage

In the two preceding chapters we have dealt with the characteristics of bacteriophagy and with the behavior of those bacteria which succeed in resisting the action of the principle which tends to destroy them. We have seen that this principle exists in the form of corpuscles which behave in all respects like true parasites. Nevertheless, a contrary opinion, advanced at first by Kabeshima, then adopted by Bordet and later by a number of other bacteriologists, has been expressed. For some 10 years now a discussion has been going on between those who recognize the parasitic living nature of the bacteriophage, a theory which I advanced in the beginning, and the adherents of a theory which views the bacteriophage as a chemical substance secreted by the bacteria.

In this connection it is not out of place to observe that as a matter of fact this discussion actually antedates the discovery of the bacteriophage. Expressed in slightly different terms it began immediately after Islowski had shown that the pathogenic agent of tobacco mosaic was an infravisible virus, the first of this

type to be clearly demonstrated. Beijerinck became the champion of the living nature of this morbid principle which he showed to be ultrafilterable, while a number of other biologists adopted the view that it was a substance derived directly from the tissue of the diseased plant. This discussion has persisted, with repeated recourse to the same arguments, in connection with each of the ultrafilterable viruses successively discovered since then. It has even furnished the foundation for a doctrine concerning the origin of cancer. The discovery of bacteriophage has given the discussion renewed activity. Up to now the partisans of the living nature of ultrafilterable viruses and, in the same way, those who support the contrary hypothesis have succeeded in bringing to their respective theses only indirect arguments; those subject to interpretation. They have affirmed that a given fact "harmonizes better" with one or the other hypothesis, but such facts exclude neither the one nor the other. No argument has been conclusive and each school has remained unconvinced. Now, because of facility of experimentation, the bacteriophage offers a particularly promising chance to solve the problem by bringing forward arguments no longer indirect, but those which are direct and are, therefore, valid as proof.

Before entering into a discussion of the nature of the corpuscles of which bacteriophage is composed let us first consider the arguments of those who have en-

deavored to prove that the bacteriophage is a principle elaborated by the bacterium. These arguments are, as has been stated, but a repetition of those which have been raised against the living nature of each ultrafilterable virus. The first is to the effect that the dimensions of the bacteriophage are incompatible with life. The second states that the bacteriophage can not be living since it offers too high a resistance to the action of harmful agents, in particular to antiseptics. The third objection is that bacteriophage is encountered in nature; that stock cultures of bacteria are to be found containing bacteriophage. The fourth affirms that the lytic principle regenerates exclusively at the expense of bacteria during the process of division, and, consequently, must be derived from the metabolism of these bacteria.

Of what value are these diverse arguments? As has been stated, in order to evaluate an argument in such a discussion it is only necessary to ask if the fact advanced as opposing living nature excludes life, that is, is the fact incompatible with life? I hardly believe that any reasonable person would deny that such a question may well constitute the criterion for the validity of an argument.

To grant the principle that by the sole fact of its dimensions a being belongs within the category of living beings or in that of inert chemical substances is to say that life depends upon a geometric property.

Obviously this cannot be true. Since, in the present state of our knowledge it is impossible for us to define the conditions which must be realized in order for life to result, we can, therefore, not fix a limit of minimal size which will permit the realization of these unknown conditions. Who can say where life really begins? So it is that when one discovers a new being whose behavior permits the supposition that it may be living one can not *a priori* invoke its small size as being incompatible with life. To be logical one must try to discover if among its diverse characters there is some one, or, perhaps, several attributes which can be considered "characteristic of life". If it can be demonstrated that the being in question possesses these characters the case is irrevocably settled.

Even if the observation were correct, would the fact that the bacterium is vulnerable only at the moment of division have the argumentative value which Bordet would attribute to it? Could it not be that the moment of division might be the only period during which the bacteriophage could penetrate into the bacterium because of a diminished resistance of the membrane or of the superficial layer surrounding the organism? But there is no occasion to seek for such an explanation. The observation invoked by Bordet and by his partisans, and which forms the basis of his theory, is in itself incorrect. As I have stated, young bacteria are indeed particularly susceptible, but never-

theless old bacteria will undergo a complete bacteriophagy. This observation has been abundantly confirmed by many, among whom may be mentioned even a collaborator of Bordet, Gratia, who has shown that cultures of the staphylococcus several days old and in which many of the cells are undergoing a senile degeneration, and, consequently, are certainly incapable of reproduction, undergo complete lysis through the action of a potent bacteriophage. Needless to say Bordet has always refrained from mentioning these experiments performed by his collaborator. More recently Schultz has again emphasized the fact that old cultures may undergo a complete lysis. Thus, even though the argument of Bordet would prove nothing by itself, it is based upon an inconclusive experiment and must, therefore, be abandoned.

Another argument which it is rather astonishing to encounter is that based upon the resistance of bacteriophage to antiseptics. The results of many experiments show that this resistance is, at most, equal to that of the spores of *B. subtilis* and, indeed, in the case of several antiseptics it is lower. Since a being unquestionably living, such as *B. subtilis,* withstands a harmful action how can one invoke a similar behavior in another being as proof that it cannot be alive?

As for the argument based upon the fact that strains of bacteria containing bacteriophage may be found in

nature, it has been invoked so many times as a proof of the production of bacteriophage by the bacterium itself that it warrants discussion.

We must first observe that not every bacterial strain contains a bacteriophage. The presence of bacteriophage is but occasional, and this in itself is an indication that when a bacteriophage is present it represents a "contamination" in the bacterial culture.

Bacteriophage is present in the intestinal content of every man and of every animal. It can be isolated from every material subject to contamination with excreta. Bacteriophage has been found in the blood and in the organs, particularly in the case of patients recovering from infectious disease. If, by plating, we culture these materials with a view to the isolation of a particular bacterium we spread over the surface of the medium not only bacteria, but the bacteriophage corpuscles which are often present. After incubation, if we select a pure colony of the bacterium this colony will be the origin of a pure non-mutant strain of the bacterium. If by chance we select a colony "contaminated" with bacteriophage corpuscles this "infected colony" will be the origin of a mutant strain in which bacteria and bacteriophage will continue to live together in a state of symbiosis.

We can readily prove that such a symbiosis exists. Let us take a "pure" bacterial strain and inoculate it into two tubes of bouillon. One of these will serve

as a control, and into the other let us add a drop of a suspension of bacteriophage active for this bacterium. Let us place the two tubes in an incubator at 42° C. Bacteriophagy will take place in the tube containing the bacteriophage, but soon a secondary culture will develop. Of each of these cultures, "pure" and "contaminated", let us make 50 transplants on agar. After they have grown we can easily see that the strain originating from the control is a pure, constant strain free of bacteriophage, while that originating from the secondary culture is a mutant strain from which bacteriophage can be isolated. This experiment shows clearly the origin of the "contaminated" bacterial cultures found in culture collections and affords a proof that bacterium and bacteriophage may live in symbiosis.

Symbiosis between a host and a parasite is frequent in nature, in fact every one of the innumerable symbioses which exist is but a particular type of parasitism wherein the virulence of the parasite is balanced by the resistance of the host. Symbiosis may be perpetual, as in the case of the "lichen", which represents a symbiosis between a fungus and an alga, but even in cases of this type it is easy to see that the fungus is the parasite and the alga the host, and it has been observed that under special conditions the fungus may destroy the alga. The situation in the "lichen" is comparable to the symbiosis between bacterium and

bacteriophage, and, as has been stated in the previous chapter, the behavior of *Piroplasma bigeminum,* the agent of Texas fever, affords another example.

Such symbioses are, indeed, so frequent in nature that they cannot be regarded as exceptions, but rather as a phenomenon so common as to seem to be general. In any case the possibility of experimentally creating a symbiosis in which bacterium and bacteriophage continue to live together throughout serial passages affords the proof that in every strain from which it is possible to extract a bacteriophage the latter must be a foreign being "contaminating" the culture. To advance symbiosis as an argument supporting the idea of the production of bacteriophage by bacteria forces us willfully to disregard the thousands of known symbioses contracted between host and parasite.

I do not believe it necessary to deal with certain arguments still more controvertible. One of this type may be cited merely as an example. In a recent publication Bordet writes, in connection with an experiment showing that a strain of bacteriophage did not cause bacteriophagy in a medium containing sodium citrate, "The theory of a virus cannot be reconciled with the observations relative to the rôle of calcium salts in lysis and the regeneration of the lytic principle." This is a strange statement. I had thought that calcium salts were indispensable for all living beings. But how can he advance such an argu-

ment, novel as it is, in view of the experimentally demonstrated fact that, although it may be true that certain races of bacteriophage do not cause bacteriophagy in a medium rendered deficient in calcium through the addition of sodium citrate, under the same conditions other races of bacteriophage will cause complete bacteriophagy. Of this Bordet can hardly be unaware, since he, himself, confirmed it. What becomes of this argument when it is confronted by the recent experiments of Asheshov, who, by means of a series of 10 passages in media containing increasing concentrations of citrate, succeeded in adapting races of bacteriophage which originally did not cause bacteriophagy in citrated media to lead to a complete dissolution? It is necessary to be poor in arguments to be forced to advance those of this character. Although lacking significance by itself the facts upon which the argument is based demonstrate that the theory supported by Bordet cannot be correct, for this theory does not explain the variations in the behavior of the bacteriophage in media containing citrate nor the adaptation of bacteriophage to such salts.

As a matter of fact, all of the arguments advanced by those who support the autolytic theory can as logically be interpreted in favor of the living nature of bacteriophage as in favor of the theory which they pretend to support. But on the other hand there are a number of features in the behavior of bacteriophage

whose interpretation by the autolytic theory seems so difficult that none of its proponents have attempted an explanation. Nothing is more surprising than to read a scientific paper in which one finds repeated on each page such a statement as "a 'lytic principle' is enhanced or is attenuated", where "the variability of this 'lytic principle' " is discussed, but in which the author refrains from advancing an explanation of the singular phenomena of enhancement, attenuation, and variability of a chemical substance. Similarly, why does he not explain how the single "lytic principle", or chemical substance secreted by the bacterium, is successively regenerated by such different bacterial species as *B. coli, B. dysenteriae, B. paradysenteriae,* and *B. pestis?* Why it is not made clear how this principle, produced by the bacterium, possesses a specific antigenic character distinct from that of the bacteria with which it is regenerated? Why do we not find an explanation of the mechanism whereby the bacterium acquires a resistance to the chemical substance which it, itself, secretes? But so much for the facts that the autolytic theory is unable to explain. And yet, a theory is valid only if it explains all the facts without exception, only if it is not forced to reject some. This principle of logic the partisans of the autolytic theory seem to forget.

And, finally, what is in reality the basis of this untenable theory? Is this bacteria-destroying principle,

which, according to Bordet, is produced by the bacterium, a normal or a pathologic substance? How can it be regenerated when we know that in nature there is a law, apparently general, to the effect that to a stimulus the living being responds by a reaction which tends to neutralize the effect of the stimulation? Here, on the contrary, to an excitation the bacterium replies by the elaboration of a product identical with that which caused the stimulation. To state, as has Bordet, that "bacteria susceptible to a given lytic principle undertake correlatively its regeneration, and it is due to this that lysis is indefinitely transmissible from one bacterial suspension to another" is but a begging of the question, a verbal explanaton. But it is, nevertheless, the entire basis of the autolytic theory. In the beginning Bordet explained the phenomenon as an "hereditary nutritive viciation". I called to his attention the fact that when a culture in which bacteriophagy has taken place undergoes filtration all of the bacteria are discarded, and because of this one can hardly speak of inheritance. Inheritance where there are no descendants is, indeed, unusual. Bordet seems to have been convinced and he has since avoided his first explanation of the phenomenon as an "hereditary nutritive viciation", but he has not yet formulated a new explanation to replace the abandoned theory. His new interpretation consists merely in the statement that "bacteria susceptible to a

given lytic principle are able, collectively to regenerate it". But this is not a theory, it is merely an affirmation and any theory based upon it would require proof that this statement is correct.

In reality the peculiarities of the phenomenon of bacteriophagy, the entire behavior of the principle which causes it, can only be interpreted and understood if one admits that the bacteriophage is a living being, a parasite of bacteria. But this in itself is not necessarily a proof. Cannot the problem of the nature of the bacteriophage be solved by direct proof, that is, is it not possible to provide, not merely arguments in favor of its living nature, but experimental proof?

The first question to be raised with a view to solving such a problem is this. What type of proof may be provided? The diverse phenomena known to man have been distributed into definite branches of knowledge called Sciences, each of which possesses its particular discipline, and has its own "yard-stick" used in measuring. I doubt if anyone will deny that life is a physiological phenomenon, consequently physiology must necessarily furnish the yard-stick to measure life. In other terms, to physiology we must turn if we would determine whether a being is or is not living.

Is there a single character or combination of characters which, if possessed by a being, proves beyond

question that the being is living? Probably everyone will admit that such a criterion exists, and that it is formed of two inseparably united characters possessed by all living beings. These characters are no longer present when a living being dies, nor are they exhibited by any purely chemical substance. The first of these is the power of chemical assimilation; ability to assimilate in an heterologous medium. This implies the possibility of transforming substances, heterologous with reference to the substance of the being possessing the faculty, into substance identical with that of which it is composed. The second character is the power of adaptation. This implies that the characters of a living being are not immutable but are in a state of equilibrium with the environmental medium. When the medium becomes modified one of two things will happen, the result depending upon the extent of the change. When the variation is too sudden or too pronounced, an equilibrium cannot be attained, the phenomenon of assimilation can no longer take place, and the being succumbs. With less violent variations a new equilibrium, through an adjustment of characters, may become established, thus assuring a continuation of assimilation. The being thus adapts itself and life continues.

The problem with which we are confronted, then, reduces itself to this:—Does the bacteriophage possess, or does it not possess, the power of assimilation

and the faculty of adaptation? If the answer is in the affirmative, further doubt is impossible, for the being is living by definition. For, let us observe, the classification into living beings and inert matter is but a human classification; we place within the first category all of the beings which possess the criteria of life, into the second those which do not possess them, and just as soon as it can be demonstrated that a being possesses these criteria it is irrevocably classified, so long as a different classification is not uniformly adopted.

Having, then, the question specifically stated, let us determine first whether the bacteriophage possesses the power of assimilation in an heterologous medium.

One introductory statement is essential, namely, that the aspect of the being, whatever it may be, never solves the question of its nature. Assimilation cannot be seen, but can be detected only by observation of the behavior of the being in question. To the sight, death cannot be distinguished from the cataleptic state, for example, and this is particularly true in the case of microscopic beings. Under the microscope nothing distinguishes a dead bacterium from a living bacterium, and if we would discover if it is alive it is essential to introduce it into a nutritive medium. If it multiplies, that is, if it transforms the heterologous substances of the medium into bacterial substance we conclude that it is living.

For those beings like bacteriophage, which can reproduce only at the expense of the living substance of a host we cannot demonstrate directly that assimilation occurs for it is impossible to observe their behavior outside of the being parasitized. Because of this complicating condition it may be objected that multiplication does not result from an assimilation of the parasite, but through an assimilative activity of the host. True. So true, indeed, that there is no occasion to refer specifically to infravisible filterable viruses. Might I ask how it may be proved that I am incorrect if, adopting precisely the same arguments as those advanced against the living nature of bacteriophage, I affirm that *Piroplasma bovis* is a corpuscle originating in the red blood cells of the cow? I offer this problem to them, as well as to those who support the endogenous theories of cancer. How can they prove that *Piroplasma bigeminum* is living?

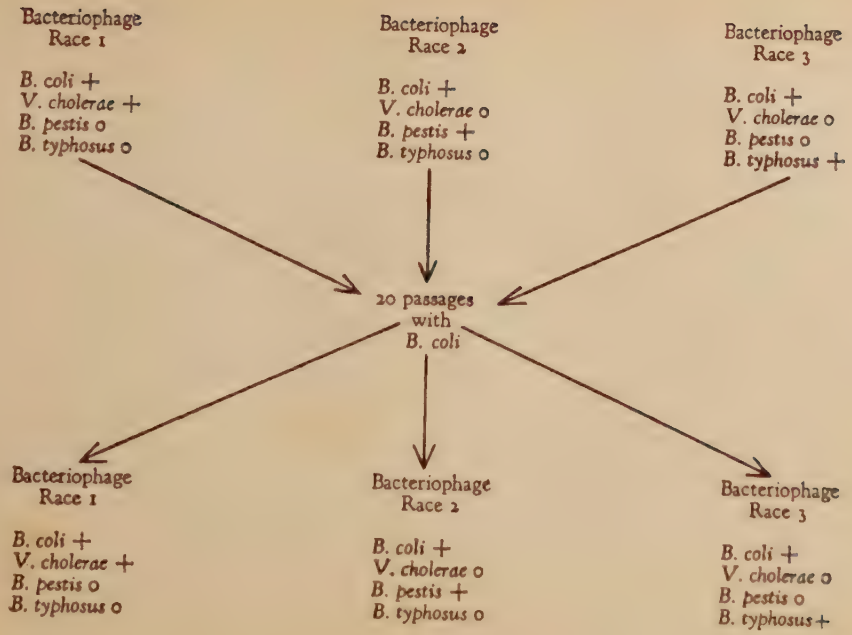
Fortunately there is another method of demonstrating the presence of the power of assimilation. This consists of demonstrating autonomy, that is, in proving that the being which multiplies and of which one seeks the nature, possesses its own characteristics, exhibiting a distinctive behavior independent of that of the host whose substance is utilized in its development. Proof of assimilation is complete if it can be demonstrated that the substance of the being in question differs from that of the host, for this neces-

sarily implies a transformation of materials during the multiplication of the being possessing autonomous characters, and this in turn implies assimilation. In the present state of our knowledge such proof is not possible for all of the obligate parasites, for example, for *Piroplasma bigeminum*, but it is, on the contrary, possible for bacteriophage.

In my published texts I have presented ten different proofs as to the autonomy of the bacteriophage corpuscle with respect to the bacterium. I will describe here only three experiments, each one by itself being conclusive. Of these three experiments I shall select two which I myself did not perform, hence their value as arguments in this connection may be considered the greater. One of them was reported by Gratia, a collaborator of Bordet; the other by Flu, and the latter is particularly striking. Needless to say, neither Bordet nor any of the adherents of his theory have ever discussed these experiments, nor have they mentioned those of the same nature which I have published. This is the more surprising since I have stated repeatedly that only experiments demonstrating the autonomy of the bacteriophage can afford proof of its nature. It would seem that when one undertakes to evaluate a theory it is but logical to consider and to try to interpret, such experiments as are specifically offered as proof of the correctness of this theory, but for five years I have begged for this discussion in vain.

Here is the first experiment. A strain of *B. coli* and three races of bacteriophage active for this single strain of *B. coli* were selected. With one of the races of bacteriophage, activity was not restricted to *B. coli* but extended also to the cholera vibrio. With a second race the associated activity was directed toward *B. pestis,* and with the third race *B. typhosus* was acted upon. With the first of these races of bacteriophage a series of 20 passages was performed at the expense of *B. coli* and at the end of this series it was shown that the bacteriophage still retained its activity for the cholera organism. In a similar way the second race of bacteriophage was carried through 20 passages at the expense of the same strain of *B. coli.* At the end of the this time it was found that this race of bacteriophage had kept its property of dissolving *B. pestis* and had not gained an activity for either the cholera vibrio or for *B. typhosus.* With the third race of bacteriophage 20 passages were made with the same strain of colon bacillus and again it appeared that this race retained its initial activity for *B. typhosus* and failed to acquire an activity for either *Vibrio cholerae* or *B. pestis.* Graphically expressed the results appear in the accompanying chart.

It is evident that if the bacteriophage were a product elaborated by the bacterium the characteristics of the bacteriophage should be determined by the bacterium itself. This cannot be the case, for each


Bacteriophage Race 1
B. coli +
V. cholerae +
B. pestis o
B. typhosus o
Bacteriophage Race 2
B. coli +
V. cholerae o
B. pestis +
B. typhosus o
Bacteriophage Race 3
B. coli +
V. cholerae o
B. pestis o
B. typhosus +
20 passages with B. coli
Bacteriophage Race 1
B. coli +
V. cholerae +
B. pestis o
B. typhosus o
Bacteriophage Race 2
B. coli +
V. cholerae o
B. pestis +
B. typhosus o
Bacteriophage Race 3
B. coli +
V. cholerae o
B. pestis o
B. typhosus +

of the three races of bacteriophage multiplying at the expense of the same bacterium retains its own distinctive characters. Thus, this experiment affords the proof that each bacteriophage is an entity. Its characters are its own property, transmissible to its descendants and completely independent of the bacterium at the expense of which it multiplies.

The second experiment is that of Gratia, who worked with two distinct races of bacteriophage. One of them caused bacteriophagy with but a single strain of *Staphylococcus albus.* Consequently, it was a strikingly monovalent race. The other race was polyvalent and caused bacteriophagy with a large number of strains of *Staphylococcus aureus, citreus,* and *albus.* These two races, therefore, possessed distinct characteristics as evidenced by their range of activity. The problem resolved itself to determining whether the bacteriophage corpuscle was an independent living being possessing its own peculiarities or whether it was an enzyme whose attributes were determined by the bacterium producing it. In other words, was the monovalent character of the first race of bacteriophage referable to a character of the corpuscle itself and capable of being hereditarily transmitted to its descendants or was in a character conferred by the *Staphylococcus albus* with which it regenerates? One of these alternatives must be true, and it is easy to repeat Gratia's experiment and ascertain which is cor-

rect. Let us make several passages of the polyvalent bacteriophage at the expense of that specific strain of *Staphylococcus albus* which regenerates the monovalent race of bacteriophage. After an indefinite number of passages we find that the character of polyvalency remains unmodified. We must, therefore, conclude that the first alternative is the correct one and that the characters exhibited by a bacteriophage corpuscle represent its own peculiar properities, transmissible to its descendants. The bacteriophage cannot, therefore, be a product of the bacterium; it must be an autonomous being.

The third experiment proving this same point is as follows: Flu isolated, from the water of a canal in Leiden, a bacteriophage which caused bacteriophagy with *B. coli, B. dysenteriae, B. paradysenteriae,* and *B. pestis.* He noted that after having been held for 30 minutes at a temperature of 58°C. this bacteriophage retained intact its property of attacking *B. pestis,* and that it had completely lost that of acting upon *B. coli.* He then made a few passages at the expense of *B. pestis* and observed that this bacteriophage had regained all of its activity for *B. coli.* This experiment again affords absolute proof of the autonomy of the bacteriophage corpuscle. It proves that the bacteriophage corpuscle possesses characteristics which are peculiar to it and not derived from the bacterium at the expense of which it reproduces. This experiment is conclusive.

We also know that antigenic character is specific, determined by the chemical constitution of the substance which provokes the formation of antibodies. The injection of an animal with pure bacteriophage corpuscles freed of all dissolved bacterial substance leads to the production of a specific antibacteriophage antibody unassociated with bacterial antibodies. It follows, therefore, that the substance of the bacteriophage corpuscle is specific, independent of the bacterial substance. Indeed, Bordet was the first to reveal the antigenic nature of bacteriophage.

The bacteriophage corpuscle possesses, therefore, autonomous characters and the substance of which it is formed differs from the bacterial substance. In order that this autonomous corpuscle may multiply at the expense of bacteria which are foreign to it, it is necessary that it transform bacterial substance into bacteriophage substance and this can take place only by virtue of a phenomenon of assimilation. Consequently, the bacteriophage corpuscle, endowed with the power of assimilation, can only be a living being, parasitic of bacteria.

Let us pass to the second characteristic distinctive of living beings, the capacity for adaptation.

Here again I first provided experimental proof of the adaptability of the bacteriophage corpuscle to glycerin and to acidity. A description of all of the

experiments conducted on this subject is entirely unnecessary to demonstrate the point at issue. I will, therefore, cite but three, each conducted by a different author.

Prausnitz passed a bacteriophage through 33 successive contacts in bouillon containing increasing quantities of phenol, making a parallel series of 33 passages with the same bacteriophage in bouillon lacking the antiseptic. After these 33 passages he took two tubes containing bouillon with 1.5 per cent of phenol and he placed in one of these tubes a drop of the suspension of bacteriophage corpuscles which had undergone the 33 passages in phenol media, while to the other tube he added one drop of the bacteriophage which had undergone the 33 passages in pure bouillon. Specimens were removed after 24, 48, and 96 hours, and by the plaque method, counts of the living corpuscles were made. He discovered that after 48 hours of contact with the antiseptic there were 2,000 living corpuscles in the case of the adapted bacteriophage, while with the bacteriophage unadapted to phenol all were dead. His results may be summarized thus:

Adapted Bacteriophage Corpuscles		*Unadapted Bacteriophage Corpuscles*	
Living after 24 hours	2400	Living after 24 hours	3
Living after 48 hours	2000	Living after 48 hours	0
Living after 96 hours	2000	Living after 96 hours	0

Prausnitz has also affected adaptations to chloramine and to mercuric chloride, as well as to an antibacteriophagic serum, succeeding with the last in obtaining a serum-resistant bacteriophage. May we note in passing that these last experiments of Prausnitz, which, by the way, I have repeated, are directly contradictory to the results reported to the effect that the neutralization of bacteriophage by an antiserum accords with the law of multiple proportions.

Munter and Rasch have repeated the experiments of Prausnitz bearing on adaptation, reporting that they failed to obtain the same results with other races of bacteriophage. This is hardly an objection to the principle in question, for even though there were but occasional races of bacteriophage susceptible to adaptation, the fact that adaptation is possible with some races is none the less established. But this is not the case. I also have repeated the experiments of Prausnitz, using the same antiseptics, with four different races of bacteriophage, and adaptation has occurred with each of them. Munter and Rasch raise still another objection, for according to them such an adaptation is purely imaginary and they suggest that the positive results of Prausnitz were due to the fact that he utilized a strain of *B. dysenteriae* contaminated by a bacteriophage. Such an objection is, indeed, singular and I am astonished that Bronfenbrenner, who has adopted it in a recent communication, and who must

moreover, be familiar with the original paper of Prausnitz, has not observed that this author's experiments were not lacking in controls. If the results which Prausnitz obtained were due to the fact that he had utilized a lysogenic bacterial strain how is it that the *same* unadapted bacteriophage, tested with the *same* bacterial strain at the *same* time and according to the *same* technic, failed to yield plaques? If the adaptation were not real the number of plaques should have been approximately the same with both the adapted and the unadapated bacteriophage.

I have succeeded in so adapting a race of bacteriophage that it would cause bacteriophagy in media having an acid reaction, a result which has been duplicated by Schuurman in my laboratory at the University of Leiden. With a strain of bacteriophage selected by chance, carried through 47 passages in media of increasing acidity, he obtained complete lysis in media having a pH of 5.6, when the same bacteriophage, unadapted, caused none at all. The following table summarizes this experiment. (Table V).

One more experiment showing adaptation may be mentioned—that of Asheshov, who so adapted the bacteriophage that it would induce bacteriophagy in media containing sodium citrate. As mentioned above, we know that while some races of bacteriophage are not affected by the addition of this salt, others, on the contrary, will not attack bacteria in its

TABLE V

Bouillon 10 cc.	Culture of *B. dysenteriae* Flexner	Bacteriophage	Result after Incubation
pH 5.8	1 drop	5 drops of the 47th passage	Clear
pH 8.2	1 drop	5 drops of the 47th passage	Clear
pH 5.8	1 drop	None (control)	Turbid
pH 5.8	1 drop	5 drops of unadapted bacteriophage	Turbid
pH 8.2	1 drop	5 drops of unadapted bacteriophage	Clear

presence. The influence of citrate is not due to a direct action, but rather because it precipitates the calcium salts in the form of the almost completely insoluble calcium citrate.

Asheshov studied three races of bacteriophage from the point of view of their capacity to cause bacteriophagy in media containing sodium citrate. Two of these races behaved in a normal fashion in spite of the addition of 1 cc. of a $N/2$ solution of citrate to 10 cc. of bouillon. The third, on the contrary, failed to act when the bouillon contained 0.5 cc. of the solution. He caused this last race of bacteriophage to undergo 10 serial passages in media containing increasing quantities of this salt (0.2 to 2 cc. of the $N/2$ solution per 10 cc.). Adaptation occurred progressively and after these 10 passages a complete dissolution was effected in media containing 2 cc. of the citrate solution in each 10 cc. of medium. In tabulated form the results were as given in Table VI.

In the experiments here described, as well as in others which need not be mentioned, the objection can not be raised that they are not clear-cut, for dissolution is complete with the adapted bacteriophage, and entirely lacking with the same bacteriophage unadapted. Most certainly Bronfenbrenner can not have read the original papers when he states: "The increase in tolerance of the phage, observed by them after adaptation, was usually so small as to be neg-

Table VI

Bouillon 10 cc.+N Sodium 2 Citrate	Adapted Bacteriophage Number of bacteria per cc.				Unadapted Bacteriophage Number of bacteria per cc.			
	Beginning	5 hrs.	12 hrs.	24 hrs.	Beginning	5 hrs.	12 hrs.	24 hrs.
0.5 cc.	300	400	10	0	300	1000	2000	2000
1.0 cc.	300	400	10	0	300	1000	2000	2000
2.0 cc.	300	750	25	0	300	1000	2000	2000

ligible." Such a criticism can only impress readers who are themselves unfamiliar with the details of the experiments performed.

As a matter of fact, might we not ask if the question of the adaptability of bacteriophage was not settled years ago, when I first demonstrated that the activity, or virulence of a bacteriophage, could be enhanced by serial passages with susceptible bacteria? This fact is now universally admitted. Such a change can only be the result of a process of adaptation.

In connection with this matter of bacteriophage adaptation one very strange argument has been advanced. Being forced to admit that such an adaptation actually occurs, certain authors have suggested that the agent undergoing the adaptation is the bacterium rather than the bacteriophage. The exact counterpart of such an argument would be the affirmation that during the process of the enhancement of virulence by a pathogenic bacterium during the course of successive passages through rabbits, the increased virulence does not represent a change in the attributes of the bacterium but rather an adaptation to die on the part of the rabbits. Death is the antithesis of adaptation. A living being which dies can not adapt itself, and in bacteriophagy a bacterium which is destroyed can not be the agent undergoing the adaptation.

These experiments admit of no doubt concerning

the faculty of adaptation of bacteriophage, and this provides us with the reason for the variability in characteristics manifested by bacteriophage corpuscles. Such variability has been observed and is admitted by all authors without exception. It can be conceived of only as a result of adaptation.

The bacteriophage corpuscle possesses, then, that group of attributes which characterize living beings. It is an autonomous being which possesses the faculty of adaptation and which multiplies at the expense of bacteria. It is to this ultrafilterable parasite that I have given the name *Protobios bacteriophagus.*

But there remains a final problem to solve. We know that with all known living beings the process of assimilation is carried out in a similar manner. The foodstuff utilized does not appear in nature already assimilated. The living being secretes enzymes which break down the gross food into its higher components, and it is among these products of disintegration that the being finds those compounds suitable for use in reconstituting its own substance. Is the process of assimilation similar in *Protobios bacteriophagus?*

From the beginning of my studies I was confronted by the problem of ascertaining whether the bacteriophage secretes a lysin, or lysins, capable of dissolving the bacterial structure. To this end I exposed a bacteriophage suspension to alcohol for a time suffi-

cient to kill the corpuscles. When this had been effected I observed that lysis of bacterial cultures could be accomplished by the addition of a small quantity of the alcoholic extract, but this lysis was not transmissible. I then concluded that this action must be referable to lysins secreted by the bacteriophage corpuscles, and that the lysins resisted the action of the alcohol which killed the corpuscles. Many authors quickly criticised this experiment, and I attempted to repeat it, but I was unable to obtain such clear-cut results. This induced me to study the question further and I found not only that the results of my first experiment were perfectly correct but also the reason for the discrepancy. Success or failure in the isolation of lysin depends upon the race of bacteriophage utilized in the experiment. Some races secrete lysin in such quantities that its extraction is easy, while, others, on the contrary, secrete it in such minimal amounts that the result is not clear-cut, and is, consequently, open to question.

Asheshov has found a race of bacteriophage, virulent for *B. paradysenteriae,* which secretes an abundance of lysin, and he has succeeded in isolating this lysin by ultrafiltration through membranes sufficiently dense to retain bacteriophage corpuscles but which permit passage of the lysin. In this connection I should mention that Bronfenbrenner has recently stated that, as the result of the passage of a bacterio-

phage suspension through a membrane, the plaques obtained upon agar are so reduced in size that they may pass undetected. I have carried out some hundreds of ultrafiltration experiments and Asheshov has performed an equal number, but in *none* of these hundreds of experiments have either of us observed the slightest reduction in the area of the plaque as compared with those produced by the same bacteriophage filtered through a Chamberland or Berkefeld candle.

The very careful experiments recently performed by Sertic, at the Institute of Hygiene at Zagreb, have settled in a particularly striking manner the question of lysin secretion by bacteriophage. In his studies he used the Asheshov strain of bacteriophage mentioned above.

Sertic inoculated a Petri dish with a culture of the susceptible paradysentery bacillus in such a way as to form four segments separated from each other by an uninoculated zone of agar. He touched a point in one of these seeded segments with a platinum wire previously immersed in a suspension of this particular bacteriophage, and after incubation he observed at this point a small bare plaque with a large areola of partially dissolved culture extending into all of the four segments. Sertic then touched with the wire the central clear plaque, washing the material off the wire in a culture of susceptible bacteria. As would

be anticipated this sufficed to induce bacteriophagy, for the plaque was in reality a colony of bacteriophage corpuscles. He thereupon removed a little of the magma of disintegrated bacteria from several places in the areola and in spite of many control experiments it was impossible to demonstrate the presence of bacteriophage. Sertic naturally concluded that the lysis taking place in the large areola could not be ascribed to the presence of bacteriophage corpuscles, but rather that it must be due to the action of a lysin secreted by the corpuscles lying within the true plaque, and from there diffusing into the surrounding agar. That this conclusion might be subjected to further proof Sertic removed, with a scalpel, some fragments of sterile agar from between the segments of inoculated medium. These portions of agar were ground up with glycerol and allowed to macerate for some time, after which the material was centrifugalized. The clear, supernatant glycerol extract contained no trace of bacteriophage whatever, as repeated experiments proved, but when a small quantity of the extract was placed upon an agar culture of the sensitive bacterium a clearing, particularly outspoken at the point where the culture had been touched, developed. These areas of lysis did not expand and did not contain bacteriophage for the lytic action was not transmissible. The activity of the extract was strictly proportionate to its dilution, being

still apparent in dilutions up to 1:200, but not beyond. As with living bacteria, the lytic action was also manifest with bacteria which had been killed by a prolonged exposure to chloroform vapor.

Sertic has also isolated lysin by ultrafiltration, it presenting the same characteristics as when extracted by maceration.

These experiments of Sertic establish in a conclusive fashion the fact that bacteriophage corpuscles secrete a lysin. From this it appears that assimilation in *Protobios bacteriophagus* is accomplished through the same agency as in all other living beings.

The fact that bacteriophage is a living being is of prime importance to biology for, aside from indicating the living nature of all ultrafilterable viruses whose nature has likewise been contested, it shows further that beings constituted not of a cell in the histological sense of this term, but by a simple protoplasmic micella may be endowed with all of the characters of other living beings: in a word, that the unit of living matter is not the cell, but the micella.

We do not yet know what life is, that is to say, as yet we have not succeeded in identifying the physicochemical property which confers upon the protoplasmic micella the faculty of assimilation. This problem, the ultimate end of biology, is not yet, certainly, ready for solution, but we may say, without fear of contradiction, that it will be some day. To

this end, attention should be directed to those living beings in which the complexity of the phenomena of life is certainly reduced to a minimum.

But—perhaps you will say—how can we study a being that we can never hope to see? To this the reply is that all studies leading to conclusions based solely on visual results can but lead to error. The entire history of human knowledge amply serves to prove this. Let us merely recall that the science of physics has made such phenomenal progress during the last few years simply because physicists have commenced to study the invisible. We may never know the electron, but we begin to understand its behavior, and this has sufficed. Throughout the physical and natural sciences the study of behavior is everything. It alone can lead to truth.

Why do not biologists decide to adopt a method which has been so successful with physicists? The effort required is not great, for they have only to study life as physiologists. Physiology is the science of behavior, without morphological considerations. It is precisely with the discipline of physiology that I have tried to resolve the problem of the nature of the bacteriophage.

CHAPTER IV

Infectious Diseases

1. *The Virulence of the Pathogenic Bacterium*

UP TO a very recent period all of the infectious diseases were attributed to anomalies which developed spontaneously in the functioning of organs, even though from the more remote times both philosophers and physicians, antedating their era, had suggested that the cause of certain diseases might be exogenous, that is, of parasitic origin. The studies of Leeuwenhoek, the inventor of the microscope, demonstrated that this was not pure imagination, but despite his disclosures the parasitic theory did not actually begin to be established until early in the 19th century. A reading of the memoirs of Jenner shows very clearly that for this great mind the agent of variola was an exogenous virus, and contagious, while that of vaccinia was of the same nature except that its virulence had become attenuated for man by repeated passage through calves.

Beginning with this period the parasitic theory gained ground rapidly. At about 1850 Villemin provided the experimental proof of the parasitic nature of tuberculosis, and at about the same time Davaine

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discovered that anthrax is caused by microscopic rods which swarmed in the blood of diseased animals. In 1868 Obermeier discovered the spirillum of relapsing fever. Shortly afterward the studies of Pasteur and of Koch established the parasitic theory upon solid grounds, while those of Metchnikoff and of von Behring served as a basis for building up the complementary theory of immunity. But in spite of these discoveries, the old endogenous theory has persisted, and, as we have seen, it is still invoked by many authors to explain the origin of diseases caused by filterable viruses. It is, indeed, difficult for man to free his mind of old errors. A century has not yet elapsed since a violent controversy disturbed academies of medicine, provoking heated quarrels between those innovators who attributed mange to the presence of an acarid, *Sarcoptis scabiei,* and those, far more numerous, who regarded it as a disease caused by a humoral disturbance, despite the fact that for some hundreds of years ignorant farmers had been able to remove the insect with the point of a needle. Except for the size of the parasite involved the same discussion continues now with reference to the numerous diseases caused by infravisible viruses.

Infectious diseases may be classified into five categories, according to the nature of the agent which causes them;—diseases caused by a pluricellular animal; those due to a protozoan; diseases caused by a

fungus; bacterial diseases; and, finally, those induced by infravisible viruses. Although the first three categories include diseases of great interest, we will consider here only the bacterial diseases and those due to infravisible viruses.

When the study of any phenomenon whatsoever is undertaken it is essential first to adopt a general method of investigation. I believe that the sole method leading to the solution of biological problems in general, and particularly problems dealing with infectious diseases, consists in accepting as a basic principle, the fact that the characteristics of living matter are the power of assimilation joined with the faculty of adaptation, and that the complete behavior of living beings originates in these fundamental attributes of living matter. Let us see if this method enables us to understand the distinctive features of the subject with which we are now concerned.

The first question to arise deals with virulence.

The great majority of bacteria are saprophytic, that is to say, their faculty of assimilation can only be exercised upon dead matter, for the enzymes which they secrete are unable to break down living material. Herein bacteria conform to the general law, for it is uniformly true that substances which are living are not attacked by enzymes. For example, we know that usually the hen's egg is not sterile when laid. It contains bacteria, and may remain without under-

going decomposition for days or even weeks. It is only after a greater or less length of time that the substance of the egg actually ceases to be living, and at this time it becomes subject to attack by the enzymes of the bacteria present, which, from that time on, multiply. But certain bacteria have acquired the property of secreting particular ferments capable of attacking living substance, and this is probably the result of a process of adaptation, as certain experiments performed with saprophytic bacteria, *B. subtilis* for example, seem to show. This property allows the bacterium to vegetate *in vivo* and constitutes virulence for the animal whose component substances are thus broken down.

The higher forms of life such as the mammal are formed of an assemblage of tissues, each tissue being built up of different elementary substances. Each bacterium becomes adapted to the secretion of enzymes attacking one or another of the elementary substances entering into the composition of a tissue, and to this is due the tendency toward localization which characterizes each infectious disease. Much is said of the tropism of pathogenic bacteria; of dysentery bacilli and of cholera vibrios, for example, having an "enterotropism" which explains their exclusive localization in the intestinal mucosa. It is difficult to believe in such tropisms, for it is hard to understand the nature of the force which directs a given bacter-

ium toward a definite organ. It is far more simple and it is more readily understood if we explain these localizations upon the basis of the simple faculty of assimilation. The bacterium introduced into the body is transported by chance, either by the lymphatics, or by the blood stream, or, finally, through the intestinal tract. Lymphatic transport is the most common form, and here the bacterium is frequently phagocytized upon a mucous surface by the leukocyte. All too often the phagocytic cell finds the bacterium to be indigestible, and bearing the living organism the mobile cell penetrates the tissues. But whatever may be the pathway of introduction, one of two things happens, either the bacterium is destroyed before it can become established, and we will shortly see through what mechanism, or it is carried into a tissue susceptible to the attack of its enzymes. In the latter case it multiplies and disease becomes manifest. Let us consider a concrete example. The cholera vibrio secretes enzymes capable of breaking down only those elements constituting the intestinal mucosa of man. When carried to any other tissue whatsoever it causes no disturbance, but if it enters into the intestinal mucosa either by way of the digestive tract or, as has been sugested by Sanarelli, by way of the blood stream, the vibrio exerts its faculty of assimilation and disease occurs.

If space permitted I should discuss the "do-every-

thing" bacteria, such as the staphylococcus, and show by examples that it is because of special characteristics acquired through adaptation that there have originated those strains of the staphylococcus associated with osteomyelitis, with furunculosis, and with pyelonephritis, and further that this adaptation consists in the development of a power to secrete enzymes capable of attacking and breaking down the elements of a given tissue.

Every bacterial species must, therefore, possess this common characteristic; the power to secrete enzymes permitting it to break down some of the substances of which some tissue is composed. From among these products of degradation the bacterium selects and assimilates those which can be reconstituted into material identical with its own substance.

But the act of assimilation itself necessitates an expenditure of energy and for this to take place it must effect, correlatively with assimilation, a disassimilation. As the result of bacterial metabolism there occurs, then, within the parasitized being, a liberation of varied substances—bacterial enzymes, diverse elementary products resulting from the degradation of the tissue attacked, products of bacterial disassimilation, and finally, bacterial substance derived from those bacteria destroyed within the body through activities which we must consider. Although all of these products originate, in the last analysis,

in the substance of the host, they are, nevertheless, foreign substances, just as, to cite an example, the muscle of the cow is different from the plant which has served to build it up. Each of the substances originating from bacterial metabolism is more or less toxic for the host, but all are "abnormal," and the body of the host *reacts* to them. The sum total of these reactions is manifest in the form of symptoms.

The metabolism of each bacterial species is effected in a distinctive manner, the products liberated differ for each species, as do the reactions opposed by the invaded organism, and, as a result, the symptoms assume a particular, specific type with each bacterial species, that is, in each disease.

On the other hand it is essential not to over-emphasize resemblance in appearance as presented by attacks of a single disease. A first-year student of medicine knows that it is often difficult to establish a diagnosis, and after he has entered upon practice he appreciates that it is even more difficult than he had believed.

Uniformity does not exist in biology, for the faculty of adaptation with which a living being is endowed causes all of the individuals within a single species to vary incessantly in different directions. To this is due the diversity which exists. If we take as an example such a clearly defined disease as typhoid fever we find that in a given culture of typhoid ba-

cilli, even though all are derived from a single bacillus, there are no two bacilli having identical characters. On the one hand, the hereditary characters are unequally transmitted, as has been shown with reference to resistance to the bacteriophage, and, on the other hand, the environment in which each organism grows differs, even within a single culture, for oxygen is more abundant at the surface, substances available for assimilation are more abundant in the center than at the surface where the organisms often form a pellicle, or at the bottom where they form sedimented masses of bacilli, and the excretory products of the bacteria accumulate where the population is most dense. All of these factors ensure that in different portions of the fluid the environmental conditions are different and each bacillus reacts to the existing conditions of the medium, adapting itself to these conditions.

What occurs in the test-tube also takes place when the bacillus grows within the body. The characteristics of each bacillus are different, the products of its metabolism differ, the reactions expressing the response of the body to those foreign substances are also different, and, consequently, the symptoms, which are the manifestations of these reactions, differ.

But this is not all, for the being parasitized is also alive. It also undergoes adaptation. There are no two men, for example, who have the same acquired

or inherited characters of resistance for the typhoid bacillus. I might further add, anticipating somewhat some of the material still to be presented, that the actions and reactions are not solely between these two beings, man and bacterium, for the bacteriophage also intervenes;—a third living being and, hence, a third variable is introduced. There can not be, therefore, a symptom-complex always the same, indicative of a given infection, but only a general resemblance of symptoms, with many instances where the resemblance is not too striking. Indeed, as Laennec has said, we do not have diseases, we have only patients. This may appear a paradox to the student, but after a few years of medical practice he will repeat with conviction the dictum of Laennec.

When placed in a medium containing substances which it can break down by virtue of the enzymes which it secretes the living cell exercises inexorably its faculty of assimilation. In the superior animal the quantity of assimilable products utilized by the cells is limited by physiological conditions, such as the digestive capacity, or even by psychic states. With inferior beings the capacity for assimilation is unlimited; it can continue without interruption to the time when the foodstuff is exhausted, provided environmental conditions, such as temperature, chemical reaction, and accumulated harmful waste products, are not inhibitory. The mass of these beings would

tend, therefore, to increase without limit if growth were not restrained by certain complex physico-chemical phenomena which ensure that when the limit of mass is attained the being divides into two like elements. Multiplication thus continues as long as the function of assimilation can operate.

In brief then, virulence is a direct result of the act of assimilation, for it represents the power possessed by, or acquired by, a bacterium of secreting enzymes capable of breaking down certain substances which enter into the constitution of a given tissue. During this process of assimilation there is, necessarily, the production of various products *abnormal* to the host, and these cause lesions of one type or another, thus leading to symptoms.

2. *Contagion*

In the absence of assimilable substances bacteria may remain alive in a latent state for a greater or less length of time. For the gonococcus and the virus of trachoma, for example, the period of latent life in the external world is very short and does not extend beyond a few hours, or even a few minutes. For other bacteria, the spore-producing forms in particular, life may persist over many decades. Provided life remains, even though it be latent, once the bacterium is restored to a suitable environment containing assimilable substances, it begins again to exercise its functions.

Of necessity all infectious diseases are to some de-

gree contagious. The bacterium multiplies in an infected host from which it is distributed into the environment, either through the death of the parasitized host with a liberation of the bacteria through decomposition of the tissues, as is the case with anthrax; or if the host survives, the pathogenic organism may be present in the excretory products and it is distributed with them throughout the environment, as is true in cholera, in typhoid fever, and in dysentery; or the pathogenic organisms may be withdrawn from the blood by a biting insect which then proceeds to inoculate another individual, as happens in plague and in typhus exanthematicus; or, again, the bacteria may be communicated by direct contact, as in the case of syphilis; or, finally, it may be transmitted from the diseased individual to its offspring in the course of reproduction, as occurs in syphilis and, without doubt, in tuberculosis also.

The facility with which different diseases are transmitted varies in accordance with a great many factors. The first of these is the mode of transmission, as we have seen. Ease of transmission also depends upon how long the organism may subsist in an inactive state, as well as upon its capacity to resist those destructive agents to which it may be exposed. Another determining factor is whether or not the pathogenic organism may assume a saprophytic existence. Again, much depends upon whether its faculty of assimila-

tion can be exercised at the expense of one or of several animal species. And, lastly, the ease with which organisms undergo attenuation is of very great importance. Let us observe that all of these factors vary, not only from one bacterial species to another, but that they likewise vary among the strains belonging to a single species. It is to this fact that two epidemics due to a single bacterium may present different characters, and that a single disease may be endemic or epidemic according to circumstances.

But this is not all, for in addition to these known factors there are certainly many unknown factors which influence contagion. All intermediary stages exist between the type of contagion seen in leprosy, a disease in which transmission is so difficult that one may live for decades in the environment of lepers without contracting the disease, and the type of contagion found in influenza, which, in 1918, became disseminated within a few months over the entire surface of the earth, attacking many millions of people. To review here the different diseases and their degree of contagiousness is unnecessary, but let us consider briefly a few particularly typical cases which reveal the extreme complexity of the question.

The tubercle bacillus possesses a high degree of viability. It is one of the bacteria most resistant to destructive agents. It may infect a great many animal species. Inoculation experiments show that it retains

its virulence for a long time. One might think, then, that tuberculosis would be an extremely contagious disease. But this is not so. Very frequently those working in slaughter-houses cut themselves with knives which have been used in cutting the tissues of infected animals. In the majority of instances nothing abnormal results; occasionally a benign cutaneous lesion develops. About 4 years ago, while opening a sealed culture tube it broke, cutting my hands. The injury was so filled with tubercle bacilli that I scraped them out with the back of a scalpel, and although I did not carry out any disinfecting procedure, nothing happened. The bacillus was, nevertheless, virulent, for guinea-pigs inoculated with it died in five weeks. We know, furthermore, that the spread of contagion from patients must often be very difficult, indeed, impossible to accomplish. Who does not know of families where one of the members contracted tuberculosis and died within a few years, while the others have not been infected despite the fact that contact with the patient was of the most intimate character? Most assuredly the tubercle bacillus requires very special conditions if it is to become implanted into a healthy host.

What are these conditions? The hypothesis that as a general rule infection must occur during the first few months of life has been advanced by von Behring. The tubercle bacillus introduced into the body

of the infant either multiplies immediately, causing thus a rapidly fatal disease, or it remains in a latent state within the host during a greater or less length of time, even for several decades, when, conditions becoming favorable, multiplication begins. The known facts render this hypothesis highly probable, and very recently additional facts have been discovered.

The tubercle bacillus may exist in two states,—in the recognized bacillary form and in the form of very fine granules, and it appears from the experiments of Valtis and of Arloing, that the corpuscular form is able to pass through the placental filter and to infect the fetus. One may well ask if this last mode of infection is not common, the organism, as in accord with the hypothesis of von Behring, remaining in a latent state within the body for an indefinite period. However this may be, we can readily see that the question of contagion in tuberculosis is complicated and is still full of uncertainties.

Let us pass to the case of a disease which is very different and which I have studied extensively, hemorrhagic septicemia of the buffalo. The causative agent is a very fragile organism. It does not withstand desiccation. It attacks only the bovine species, and it undergoes a rapid attenuation. One might reason that the disease caused by this organism should be but slightly contagious but, on the contrary, it is one of the most contagious diseases known. I have wit-

nessed an epizoötic, which, within the space of one month, spread throughout a region of several thousand square miles and caused the death of a half of the cattle found there. This region, in the eastern part of Cochin-China, is, moreover, very thinly settled; it is totally lacking in roads, and communication between the different villages, very remote from each other, is difficult and infrequent. The disease is caused by a bacterium belonging to the Pasteurella group. Infinitely small quantities of cultures of this organism injected into healthy animals cause a disease identical, as to lesions and symptoms, with the natural disease, proving thus beyond question that the Pasteurella is the specific agent. But the strange feature is that the experimentally produced disease is never contagious, whatever may be the measures taken to favor its spread. How is contagion effected in the natural disease? This is a mystery.

But this peculiar behavior as regards contagion in hemorrhagic septicemia is not an isolated instance, for the same problem is encountered in influenza and, in general, in all epidemic bacterial diseases. In all of them the natural disease is contagious, the experimental disease is not. There is, therefore, justification for the belief that the cause of contagion is to be found outside of the bacterium.

In this connection it is always well to bear in mind the admirable studies of de Schweinitz and Dorset

who demonstrated that hog cholera is not due, as had been believed, to a bacterium of the Salmonella group, but rather to an infravisible virus. As a matter of fact, hog cholera is a complex disease which may be caused either solely by the visible bacterium, the Salmonella organism, which is often encountered living as a saprophyte in the intestines of healthy swine, and in such cases the disease is not contagious; or solely by the infravisible virus, and then the disease is extremely contagious, or, finally, and this is the most common, by the two organisms together where their pathogenic effects are superimposed. It is probable that the situation is the same for other epidemic diseases, and one might ask if the infravisible virus may not be the filterable form of the visible bacterium. Some years ago I advanced the hypothesis that bacteria are not cells, in the histological sense of the word, but are aggregates of corpuscles, each of which is endowed with elementary life and is capable, under certain conditions, of multiplying in the dispersed state, and I also called attention to the possible rôle of these corpuscles in contagion. The recent discoveries in tuberculosis suggest that this was not purely imaginary.

Let us pass to a third disease, which I have also studied, in which the factors which contribute to contagion are still different. This is cholera. At first sight the question of contagion would appear very simple.

The cholera vibrio develops in the intestines of man and leads to an attack of the disease. The vibrios are distributed throughout the environment with the excreta of the patient and are then further disseminated, either directly by potable waters or by the intermediary of flies which contaminate the food ingested by susceptible individuals. These persons then contract the disease, and the cycle begins again. As a matter of fact the question is far more complicated.

In regions where cholera is present individuals affected with a simple diarrhea, without other symptoms, are common. But in spite of the fact that these persons continue about their daily tasks, and are not seriously ill, the intestinal disturbance is caused by the vibrio, and the organism is multiplying within the intestine. Manifestly, the presence of vibrios within the intestine is not the sole requisite for the development of cholera. Is it because these individuals are refractory? By no means. This is proved by the fact that very frequently this simple diarrhea, after a variable latent period, which may reach 15 days, becomes transformed into an attack of cholera, all too frequently fatal. It is necessary to believe, then, and there is other evidence supporting this idea, that cholera manifests itself only if the vibrios are able to grow in the intestinal mucosa, that is, only when they are able to assimilate and to develop at the expense of the substances constituting this mucosa.

But let me mention a fact still more baffling. We have seen that in hog cholera and in hemorrhagic septicemia of the water-buffalo it is possible to produce, with the organism incriminated, an experimental disease comparable to the natural infection aside from the fact that it is not contagious. Experimental cholera infection is impossible. Since the discovery of the vibrio more than a hundred bacteriologists have ingested cultures of this organism without experiencing any disturbance. In one instance it is true, one observer mentioned that a benign attack of cholera was produced by the ingestion of a culture, but the attack was so mild that one might well question if the imagination of the investigator did not play a part in this result. In Spain, Ferran, under the pretext of vaccination, has inoculated more than 300,000 individuals with *living cultures* of the cholera vibrio and in no case did an attack of cholera occur. Must we conclude that the vibrio is not the real agent of cholera? Not at all, for everything shows that it is responsible for the disease, and the studies which I have carried out in India during the past year reaffirm that only this vibrio is responsible. All of the peculiarities of the disease and of its contagion rest upon the inherently variable characters of the virulence of the organism.

Thus, we have here three bacterial diseases, for each of which the distinctive conditions governing

contagion seem to be different. But the subject is not yet exhausted, for, as I have remarked above, the conflict is not in reality solely between the bacterium and the superior host. A third living being always comes into play. This is the bacteriophage, the parasite of bacteria, and it constitutes a third factor just as variable as are the other two.

The three diseases which we have taken as examples are contagious to different degrees, but there are other diseases which, although due to bacteria, do not appear to be so. Such are the different infections in which the agent is either *B. coli* or the staphylococcus; such conditions as pyelitis, pyelonephritis, cholecystitis, osteomyelitis, etc. All of these diseases are chronic conditions, and when the bacteria isolated from the lesions are examined, it is found that in all cases the agent is not an ultrapure bacterial culture, but, according to the case, a symbiosis between the colon bacillus and bacteriophage, or between the staphylococcus and bacteriophage. This observation becomes still more significant when we note that certain other bacteria, such as *B. typhosus* or *B. dysenteriae,* agents of acute diseases, may likewise cause chronic infections such as cholecystitis, and that in these cases the bacillus isolated at the beginning of the acute disease is ultrapure while that found in the chronic condition has established a symbiotic relationship with bacteriophage. It appears, then, that while

the acute diseases are caused by ultrapure bacteria the chronic conditions are due to symbioses between bacterium and bacteriophage. In making this general statement, even tuberculosis is not excluded, for I have reason to believe that the forms which we call tubercle bacilli represent in reality a symbiosis between a bacterium, as yet unknown in the ultrapure state, and a bacteriophage. But however this may be, by virtue of the symbiosis with a bacteriophage the virulence of the pathogenic bacterium becomes considerably attenuated. This, many authors have observed, and as I have noted with the cholera vibrio, all virulence becomes lost under these conditions.

Consideration of the question of contagion shows that in principle all of the infectious diseases are contagious, but the degree of contagiousness varies in accordance with a number of factors, several of which are as yet unknown. Among the factors of greatest importance in governing contagion is the influence of the bacteriophage. This aspect of the problem will be considered in a later chapter, wherein its relationship to the spread of infectious disease and to many of the obscure features of contagion will be discussed.

3. *Immunity*

Let us turn to another aspect of the problem of infectious disease which, as yet, we have considered only as it relates to the bacterium. How does the body of

the invaded being behave? One of two things happens. Either the being dies or it recovers. The first case does not interest us, and while later we will discuss the reason for the recovery, let us at the moment consider how the cured individual behaves.

From the most remote times it has been known that certain diseases do not recur or, at least, second attacks are extremely rare. This is true for variola, for plague, and for typhoid fever among others. Other diseases, on the contrary, do recur and, indeed, with some of them an initial attack seems rather to render the body more susceptible to infection. Among such recurrent diseases may be mentioned pneumonia, dysentery, and the bronchial infections. There are, therefore, immunizing diseases and others which are not immunizing. What can be the cause of this difference?

It is well, at first, to recall the nature of the organisms involved. In a text published in 1924, "Immunity in Natural Infectious Disease," I showed that the immunity acquired to an ultravirus involved a process entirely different from that responsible for the immunity acquired to bacteria. Inasmuch as the processes themselves are basically different it is not peculiar that all of the diseases caused by filterable viruses should be immunizing, while this is far from being generally true for the bacterial diseases. Let us first examine the situation in diseases of the latter type.

Within a given group of bacteria we find that some species cause diseases that are highly immunizing, such as typhoid, and others cause diseases such as dysentery, to which no immunity develops. Nevertheless, *B. typhosus* is very closely related to *B. dysenteriae*. This simple fact shows us that the question must be highly complex and it is at present very obscure. All that one may assume is that the nature of the tissue or of the organ within which the bacterium grows must be one of the determining factors of the phenomenon. Septicemias are, as a rule, immunizing diseases. Diseases in which the bacterium attacks the lung or the intestinal mucosa are usually not immunizing. Another factor must be the nature of the products of the bacterial metabolism, and this factor may well be of chief importance, as we will see shortly when we consider antitoxic immunity.

What can be the cause of the failure of certain bacterial diseases to recur? This problem has stimulated many investigations, thanks to which the science of Immunology has been developed.

The first studies worthy of attention are those of Metchnikoff who devoted his life to building up his theory of phagocytosis. According to him immunity must be due to a specific property of certain cells of the body whereby bacteria are engulfed and digested. These cells, designated by the generic name of phagocytes, are either free in the fluids, as are the leuko-

cytes, or fixed in the tissues, as are the clasmatocytes of Ranvier, and especially the endothelial cells of the blood capillaries and lymphatics whose fundamental phagocytic rôle has been revealed by the excellent contributions of the American school. The phenomenon of phagocytosis is adequate to explain natural immunity, that is, the refractory state enjoyed by all members of a given animal species for a bacterium pathogenic for another species. In other words, it is an hereditary immunity; a bacterium is or is not pathogenic for an animal species depending upon whether this bacterium is or is not *engulfed and digested* by the phagocytes of this species. Let us observe here again that all natural hereditary immunity is based upon the phenomenon of assimilation, for if the phagocyte is able to exercise its faculty of assimilation at the expense of the bacterium there is a natural immunity; if the bacterium assimilates at the expense of the phagocyte there is no immunity.

Does the theory of phagocytosis explain acquired immunity? Metchnikoff believed so. In his opinion, during the course of a disease the phagocytes became adapted to the digestion of the pathogen involved, and he performed countless experiments in an effort to substantiate this theory. But even the most demonstrative of these experiments proved nothing, for they were carried out according to a method of study still dear to bacteriologists and which can not be criticised

too strongly. This method consists in taking a so-called "laboratory animal," inoculating it with a culture of the pathogenic agent of a disease to which the animal is completely refractory, and observing the result. Upon such experiments a false science has been constructed—a "laboratory immunology"—which has nothing in common with actual immunity as it exists under natural conditions.

In the case with which we are concerned the phenomena so induced do not lead to an acquired immunity but to a *reinforced natural immunity.* It would seem that this fact is sufficiently obvious, nevertheless scientific periodicals continue to be encumbered with communications describing experiments made according to such a method. The single rational method of investigation is to study natural disease; all other methods lead to false conclusions.

In order to discover whether phagocytosis intervenes as a basic phenomenon in acquired immunity it is necessary to ascertain the behavior of the phagocyte in natural disease. But we know from simple observation that diseases caused by the pyogenic bacteria, those in which the phagocyte exhibits the strongest positive chemotaxis, are all recurrent diseases. Infections due to the gonococcus and the staphylococcus may be mentioned as examples. The phagocyte is not, then, capable of adapting itself to digest these bacteria. In the experimental animal the case of the

bacillus of swine erysipelas is, indeed, curious. The horse is refractory to this disease, its phagocytes readily digest the bacterium, and very quickly after the injection of a culture all of the bacilli have disappeared from the blood. But the situation is quite different with horses which are being used for the production of an antiserum. Even when the horse is hyperimmunized, living bacilli may be recovered from the blood three and four days after the injection, as I have repeatedly observed. Instead of undergoing an adaptation, the phagocytes of the horse appear to lose their faculty of ingesting these bacilli, but disease does not result.

In reality, phagocytosis explains *natural* immunity; an animal species is refractory or susceptible to a given disease depending upon whether the phagocytes are or are not able to digest the pathogenic bacterium.

Following the discovery of phagocytosis another phenomenon was revealed by the work of von Behring and Kitasato. They demonstrated that if an animal were injected with a bacterial toxin the blood of this animal acquired the property of neutralizing the toxin. It has since been found that this is not merely an experimental phenomenon but that in natural disease also, the fluids acquire the specific antitoxic property. After an attack of diphtheria, for example, the fluids of the recovered individual neutralize diphtheria toxin. Antitoxic properties may even

be acquired without outspoken disease, for we know that antitoxin is to be found in the blood of many individuals who have not suffered from diphtheria. Similarly we find, especially in the Far East, that the blood of many Chinese has the power of neutralizing tetanus toxin. This is probably due to the fact that these individuals frequently absorb tetanus spores, as is indicated by the fact that in several instances tetanus bacilli have been isolated from the intestinal contents.

Here we have, then, an objective phenomenon. The body may acquire the power of neutralizing a bacterial toxin and retain this power for a very long time. Can all acquired immunity be fully explained by this phenomenon? The situation is extremely complicated.

As a matter of fact we are thoroughly conversant with but three toxins—botulinus, tetanus, and diphtheria. In the case of botulism there can be no doubt but that antitoxic action fully explains acquired immunity, for the pathogenic bacterium is devoid of virulence for man and disease results from the ingestion of the toxin with contaminated food. In tetanus the condition is slightly different, for in this disease the toxin is produced within a tissue, and the virulence of the pathogenic bacterium is so low that special conditions, such as the presence of a foreign body, are requisite to multiplication. Even in fatal cases the organisms often disappear. The virulence is so

low that it seems negligible and in acquired immunity antitoxic action is the sole mechanism involved.

In diphtheria the situation is entirely different. Here, the pathogenic microorganism possesses both virulence and toxicity, and quantitatively these two properties are not constant. Indeed, they are highly variable, some strains of diphtheria bacilli being highly toxic but lacking in virulence, others are of great virulence and of but low toxicity.

This condition may be expressed in another way. If we allow V and T to represent virulence and toxicity respectively, and if we indicate the lack of a property by 0 and its presence to the highest degree by ∞ we may say that under natural conditions all intermediaries between V^0T^∞ and $V^\infty T^0$ may exist. In such terms the well-known No. 8 strain (Park and Williams) of diphtheria bacilli would correspond to the expression V^0T^∞, while other strains derived from cases showing rapidly spreading membranes would approximate $V^\infty T^0$.

That the injection of diphtheria antitoxin is not always attended by a successful result is a matter of common observation, and those cases presenting the most extensive local lesions are the ones in which failure is most common. This fact can not but lead to the conclusion that although antitoxic immunity plays an important rôle in this disease, it does not explain all phases of acquired immunity. There must

also be operative within the body a process leading to the destruction of the "virulent enzyme" which, as has been stated before, breaks down some constituent of the tissues thus permitting the microorganisms to exercise their faculty of assimilation.

The mechanism of the "virulicidal" immunity is still unknown; all that may be inferred is that some "immune substance" capable of destroying the "virulent enzymes" must be secreted by those cells susceptible to these enzymes. Just as soon as the "virulent enzymes" are neutralized or destroyed, the virulence of the microorganism becomes abolished, and its elimination by phagocytosis is then effected in the same way as is the elimination of bacteria which are naturally avirulent.

We will not discuss the purely imaginary humoral theory of acquired immunity. The so-called antibacterial antibodies (agglutinin and sensitizer) exert no harmful effect upon bacteria, either *in vivo* or *in vitro*. All of the experiments upon which this humoral theory is based are experiments loosely interpreted or experiments poorly performed. My criticisms of it were published more than five years ago in the text "Immunity in Natural Infectious Disease," and none of the supporters of the theory have protested, or even attempted to refute my arguments. The antibacterial antibodies are the indices of infection and their influence, far from providing an immunity, results in a sensitization of the individual. These

anti-bodies are the price paid for immunity.

As for immunity to the so-called filterable viruses, we know that with but few exceptions the diseases (smallpox, poliomyelitis, measles, etc.) caused by these agents, for which I have proposed the generic name of Protobe, are highly immunizing.

In the text referred to above, my concept of the immunity acquired to Protobes, the intracellular parasites, is explained. The cell which resists invasion acquires an immunity; reacting to the virus, it produces an "antivirulin" which specifically neutralizes the virus. An excess of antivirulin passes into the body fluids, where its presence can be demonstrated. Experiment has shown that the antivirulin is definitely different from the sensitizing principles, for it acts without the intervention of complement. It resembles antitoxin, but that the two principles are not identical is evident from the fact that antitoxin reacts with toxin according to the law of the mass action, while "antivirulin" action does not conform to this law. Furthermore, the Protobes are able to adapt themselves to the harmful action of the antivirulin and thus become "serum-fast." This concept of the nature of immunity acquired to Protobes—the so-called filter-passing viruses—has since received strong support through the work of Schultz and his co-workers.

To summarize:—natural immunity can be adequately explained upon the basis of phagocytosis; that immunity which is acquired to bacteria is in part anti-

toxic immunity, supplemented by a "virulicidal" process of some sort; that acquired to the Protobes is completely explained as a cellular immunity effected through the secretion by the resisting cells of specific "antivirulins."

There is one last question worthy of consideration. Whatever may be its mechanism, at what moment is acquired immunity manifest? One might immediately be inclined to reply that it precedes convalescence. But certainly this cannot be correct, for how shall we explain the recovery brought about in recurrent diseases and, on the other hand, how could we interpret the relapses which take place several days after the beginning of convalescence? Instead of being benign these relapses are often fatal. It is, moreover, significant that these relapses occur during convalescence from the highly immunizing diseases, such as typhoid fever. Obviously, at the time of relapse immunity cannot have been established. Recovery cannot, therefore, be considered as due to a phenomenon of immunity, but the immunity, on the contrary, must be derived from the recovery. To what, then, can we attribute the phenomenon of the recovery?

In the next chapter we shall see that several of the problems which still serve to render obscure our knowledge of infectious diseases, principally those relating to contagion and recovery, can be explained by the intervention of a third factor. This is the bacteriophage.

CHAPTER V

Recovery and Immunity

MANY of the infectious diseases are immunizing diseases; infections in which an initial attack confers upon the body a refractory state of greater or less duration. This fact necessarily means that one of the results of the conflict between parasite and host is the acquisition of an immunity. But when does this immunity become effective? Does it develop before or after recovery? When immunity is acquired is it the cause of recovery or is it a result of recovery? This is the sole aspect of acquired immunity to be considered here, and as has been stated in the last paragraphs of the preceding chapter, we can only conclude that immunity is consequent to recovery. To what, then, can we attribute the phenomenon of recovery? This is the problem at present under consideration. But let us first restate, briefly, the characteristics of the phenomenon of bacteriophagy.

Let us take the stool of a patient recovering from an intestinal disease, such as dysentery, typhoid fever, or cholera, emulsify a fragment of this stool in water, and then filter the suspension through a porcelain

filter (Berkefeld or Chamberland). The clear, filtered fluid contains a principle which has the property of destroying and dissolving *in vitro* cultures of the specific bacterium responsible for the disease present in the patient. If, for example, we introduce a trace of the filtered stool of a cholera convalescent into a fairly turbid culture of the cholera vibrio this culture will become perfectly clear after two or three hours; all of the vibrios will have become dissolved, apparently just as sugar is dissolved in water. If, then, we introduce a trace of this limpid liquid into a second turbid culture of the vibrio the same phenomenon of dissolution takes place, just as promptly as in the first instance. If a trace of this second limpid fluid is added to a third culture of the vibrio destruction and dissolution again results, and this operation can be repeated indefinitely without the phenomenon of dissolution exhibiting any reduction in rapidity or in intensity.

The same phenomenon of serial dissolution will take place if stool filtrates from a convalescent from bacillary dysentery act upon cultures of the dysentery bacillus, or if those derived from a typhoid fever convalescent operate on cultures of typhoid bacilli. But the phenomenon is not restricted to the action of stool filtrates and in a disease such as bubonic plague, dissolution of cultures of the plague bacillus can be induced by material derived from a bubo of a plague

convalescent. The same result can also be obtained when pus derived from a furuncle or a carbuncle approaching healing is combined with cultures of the staphylococcus.

Thus, this phenomenon of bacterial dissolution takes place indefinitely in series. This can only mean that in the intestine or in the lesion there exists a principle—bacteriophage—which reproduces as the bacteria are dissolved.

We have already discussed the behavior of the bacteriophage under experimental conditions, and however interesting such a subject may be, it can only be of theoretical importance. Theoretical investigations upon this subject can only be really interesting if they are considered as an introduction to the study of the behavior of the bacteriophage in its natural environment.

Let us consider first, then, the normal habitat of the bacteriophage. I have demonstrated its presence in all beings provided with a digestive tract where it grows in symbiosis with the bacteria of the normal intestinal flora, with *B. coli* in particular. This conclusion, based upon many studies involving man, mammals, and insects, has been questioned by several authors who have been unable to isolate bacteriophage with regularity. But these authors have adopted an entirely inadequate method, they have simply prepared filtrates from stools and have tested

these filtrates upon cultures of any strain of *B. coli* whatsoever. Surely by this method only rarely can a bacteriophage be revealed in a normal stool. The reason is simple indeed. Each strain of *B. coli* behaves, with regard to bacteriophage, like a distinct bacterial species. A given strain of *B. coli* may be susceptible to the action of a given bacteriophage and totally insensitive to other races of bacteriophage which, nevertheless, strongly attack other strains of *B. coli.* This is not astonishing for, from the antigenic point of view also, each strain of this bacterium behaves as though it belonged to a particular species. Because of this diversity of action the only rational method of attempting to disclose bacteriophage in a normal stool consists in causing the filtrate obtained from this stool to act upon a culture of the strain of *B. coli* isolated from the same specimen of stool. That is, it should be tested against the strain with which it has been developing in the intestine. Consequently, to isolate a race of bacteriophage from these stools, a large number of colonies, at least 50, should be selected and the filtrate should be tested with each of these cultures. This is the only method which permits us to demonstrate the constant presence of bacteriophage in the intestine.

Being constantly found in the stools it is but a logical deduction that it must also be present in everything which in nature is subject to fecal contamina-

tion—soil, water, and the particles of dust floating in the air. This is confirmed by experiment. *The opportunity for all living beings to become "contaminated" by different races of bacteriophage is, therefore, continuous.*

Each race of bacteriophage (the term "race" is used here in speaking of bacteriophage and "strain" in speaking of bacteria merely to avoid repetition and possible confusion, the two terms having an identical significance) possesses distinctive characteristics, the chief of which is its individual behavior with respect to its action upon different bacterial species. One race of bacteriophage possesses a virulence for only certain strains of *B. coli,* a second may possess, in addition, a virulence for *B. typhosus* or *B. dysenteriae,* or for both. Along with its virulence for certain strains of *B. coli,* another race may attack *B. pestis* or the cholera vibrio, and still another may be virulent for staphylococci and certain strains of *B. coli.* Each bacteriophage that may be isolated in nature possesses a "mosaic" of virulences and each of these virulences may vary in degree. This diversity in attributes can only originate in the faculty of adaptation which, as we have seen, is extremely pronounced with the bacteriophage. Evidence of this is provided by the fact that even *in vitro* it is possible to adapt a bacteriophage to the attack of a bacterium for which it was previously inactive. This acquisition of new viru-

lences has been confirmed by several authors. We must, therefore, assume that the same phenomenon of adaptation can occur in nature and that a bacteriophage which is growing at the expense of a bacterial species may adapt itself more or less readily, according to the conditions of the moment, to parasitism of another bacterium introduced into the same environment. Let us see if this deduction can be confirmed by observation of natural processes.

The bacteriophage is not to be found in the intestinal tract of the fetus or in that of the newborn child. It makes its appearance there between the fourth and eighth day after birth, and persists thenceforth throughout life. Being widely distributed throughout nature, it is not surprising that the infant quickly becomes "contaminated."

Let us now see how the bacteriophage behaves. For a year I followed the variations in virulence of the intestinal bacteriophage of a single individual. During the course of this year 23 examinations were made, one every 15 days. Eleven of the examinations showed that the virulence was not restricted to *B. coli,* but extended to other bacteria as well—dysentery, paradysentery, and paratyphoid bacilli, chiefly. The virulence of the intestinal bacteriophage thus varies continually. While for *B. coli* it is habitual, it extends, at one time or another, to other bacteria, but the significant point is that in the course of this year on

two different occasions the person under examination experienced mild intestinal disturbances. The first of these appeared on July 3 and consisted of three of four fluid stools, with the normal state being restored on the next day. Examination of the stool specimen obtained on the 4th of July (the day following the diarrhea) revealed a bacteriophage with a very high virulence for *B. paradysenteriae.* This virulence was maintained throughout the 5th, 6th, and 7th of July, but on July 8 only the virulence for *B. coli* remained.

The second intestinal disturbance developed on the 26th of September, a few hours after a meal somewhat open to suspicion. In the evening violent intestinal pain developed with a profuse diarrhea, suggesting the presence of an intestinal infection. The next day, pain had ceased, although there were two fluid stools, and on the 28th of September the person had become normal. A specimen of the stool collected on the 27th of September showed very high virulences for *B. coli* and for *B. paratyphosus B.* These two virulences persisted up to October 3, but on the 4th the virulence was limited, as usual, to *B. coli.*

In similar experiments carried out upon many persons living in different countries it has been found that every time a transitory intestinal disturbance develops in an individual, within a few hours the intes-

tinal bacteriophage manifests a high virulence for some pathogenic organism; usually for some member of the dysentery or paradysentery, typhoid or paratyphoid groups.

Another point, still more significant, may be mentioned. In all of my studies made in Europe, in America, or in Africa, regions which are free of cholera, never have I demonstrated the presence of a bacteriophage possessing any virulence whatsoever for the cholera vibrio. But the same person in whom I had previously studied the behavior of the intestinal bacteriophage throughout a year, went to India last year (it is of myself that I speak) and was continually in contact with cholera patients, and daily worked with their excreta. One night he experienced violent intestinal pain, with weakness, sweating, and profuse diarrhea. The next day the disturbance had disappeared and a stool examination made upon this day disclosed a bacteriophage with a high virulence for the cholera vibrio.

These observations suggest an hypothesis. We must of necessity grant that the intestinal disturbance originates in the growth of pathogenic bacteria. It is equally true that the abnormal state thus produced is but transitory. Is it not possible that the normally present intestinal bacteriophage acquires a virulence for the pathogen undergoing implantation in the intestine, causing it to be quickly bacteriophaged and

destroyed, thus aborting the disease at its beginning? In other words, cure through bacteriophagy is effected with the first symptoms. In order to evaluate this hypothesis properly let us examine the behavior of the intestinal bacteriophage during the course of outspoken disease.

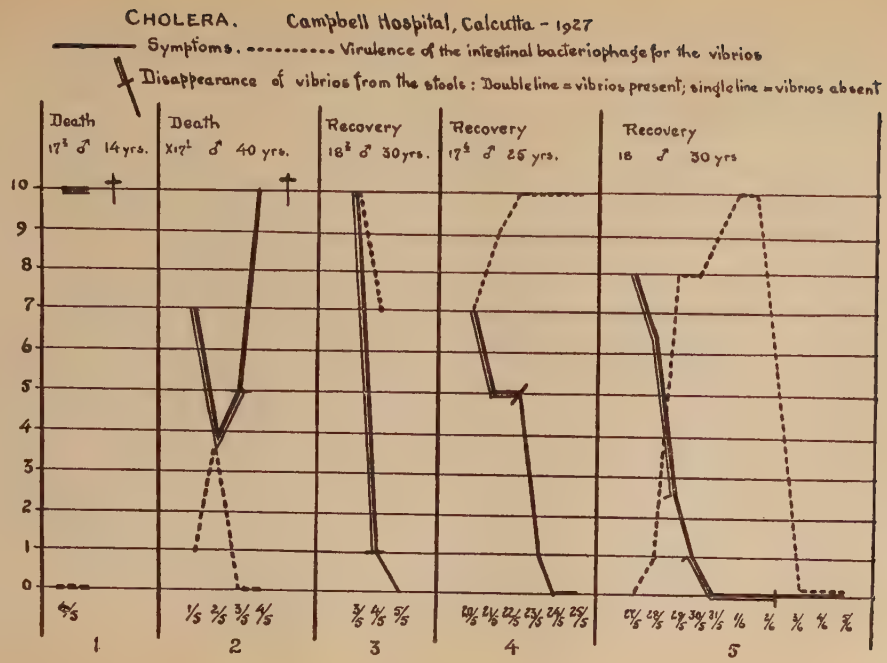
My studies were directed first to bacillary dysentery, and later to typhoid fever, but since my observations relative to these two diseases have already been published I will consider here more particularly the studies carried out during the past year upon Asiatic cholera. This will be fully as satisfactory since the behavior of the bacteriophage is identical in the three diseases.

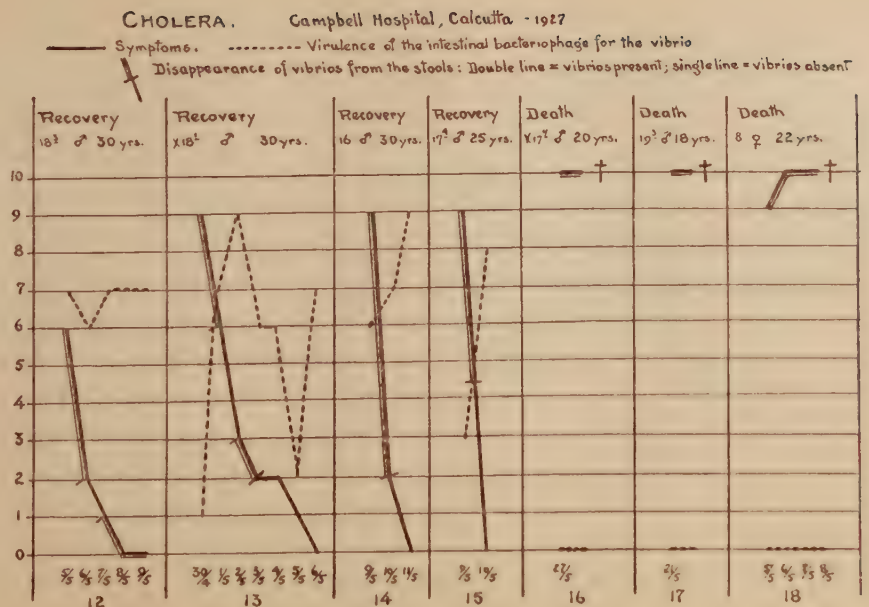
The studies carried out have been recorded in charts which show, better than a great deal of explanation, the results of the studies made on each individual case and permit a comparison of the different cases. In these charts the solid line indicates the condition of the patient as determined by the obvious symptoms. In order to express correctly the severity of the condition of the patient at each stage of the disease I have established a coefficient for each of these symptoms (number of rice-water stools, vomiting, density of the blood, cramps, collapse, anuria, pulse, general condition) and the sum of these coefficients gives a figure which serves as an index of severity. In this scale the minimum is 0, corresponding to the absence of all

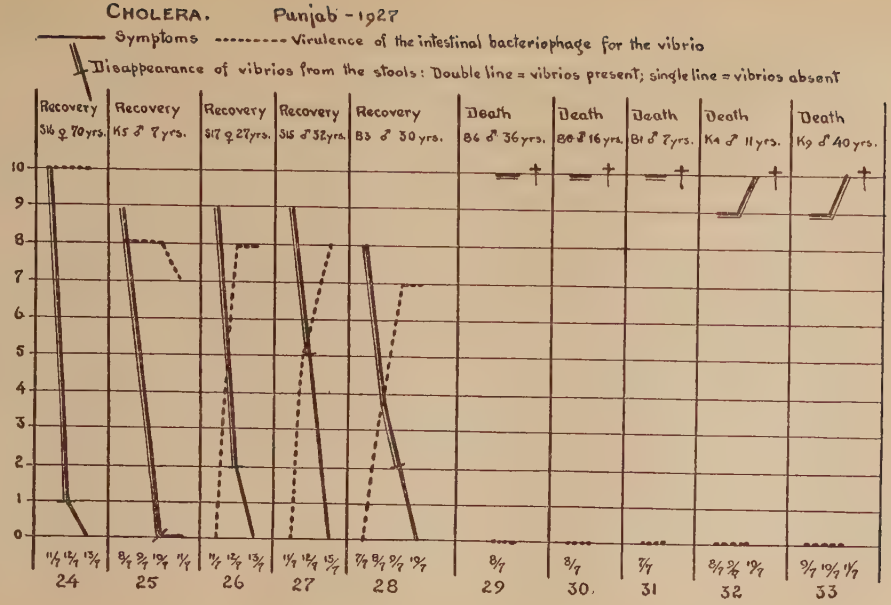
symptoms, that is, to convalescence; the maximum is 10 and corresponds to the most serious state, with all symptoms present to a maximum degree. The short, transverse line cutting the line expressing the symptoms indicates disappearance of vibrios from stools.

The dotted line shows the virulence of the intestinal bacteriophage for the cholera vibrio. We know that the virulence of a bacteriophage for a given organism is not constant; it may be hardly appreciable or it may be so high that all of the bacteria present in a culture are destroyed within a few hours. Between these two extremes is to be found a range of virulences of all degrees. A bacteriophage of maximum virulence, corresponding to 10 in the scale, causes, within a period of two and one-half hours, a complete and permanent dissolution of all vibrios present in a culture. The zero of the scale represents a complete absence of virulence for the cholera vibrio.

In every patient studied, the first examination was made within from 8 to 15 hours after the appearance of the first symptoms. Later examinations were then made, at least once in every 24 hours, up to the complete disappearance of all symptoms and even, when it has been possible, throughout the convalescent period. The distrustful character of the native renders these prolonged observations very difficult, for if the patient is taken care of in his home immediately after he is able to sit up he refuses all examinations. Those


CHOLERA. Campbell Hospital, Calcutta - 1927
Symptoms. ---------- Virulence of the intestinal bacteriophage for the vibrios
Disappearance of vibrios from the stools : Double line = vibrios present; single line = vibrios absent
Death
17½ ♂ 14 yrs.
Death
X17½ ♂ 40 yrs.
Recovery
18½ ♂ 30 yrs.
Recovery
17½ ♂ 25 yrs.
Recovery
18 ♂ 30 yrs
10
9
8
7
6
5
4
3
2
1
0
4/5
1/5 2/5 3/5 4/5
3/5 4/5 5/5
20/5 21/5 22/5 23/5 24/5 25/5
27/5 28/5 29/5 30/5 31/5 1/6 2/6 3/6 4/6 5/6
1
2
3
4
5


CHOLERA. Campbell Hospital, Calcutta - 1927
Symptoms. Virulence of the intestinal bacteriophage for the vibrio
Disappearance of vibrios from the stools: Double line = vibrios present; single line = vibrios absent
Recovery
18♂ 30 yrs.
Recovery
X18♂ 30 yrs.
Recovery
16 ♂ 30 yrs.
Recovery
17♂ 25 yrs.
Death
X17♂ 20 yrs.
Death
19♂ 18 yrs.
Death
8 ♀ 22 yrs.
10
9
8
7
6
5
4
3
2
1
0
12
13
14
15
16
17
18


CHOLERA. Punjab - 1927
Symptoms
Virulence of the intestinal bacteriophage for the vibrio
Disappearance of vibrios from the stools: Double line = vibrios present; single line = vibrios absent
Recovery
S16 ♀ 70 yrs.
Recovery
K5 ♂ 7 yrs.
Recovery
S17 ♀ 27 yrs.
Recovery
S15 ♂ 32 yrs.
Recovery
B3 ♂ 30 yrs.
Death
B6 ♂ 36 yrs.
Death
B6 ♂ 16 yrs.
Death
B1 ♂ 7 yrs.
Death
K4 ♂ 11 yrs.
Death
K9 ♂ 40 yrs.
10
9
8
7
6
5
4
3
2
1
0
11/1 12/1 13/1
24
8/1 9/1 10/1 11/1
25
11/1 12/1 13/1
26
11/1 12/1 13/1
27
7/1 8/1 9/1 10/1
28
8/1
29
8/1
30
7/1
31
8/1 9/1 10/1
32
9/1 10/1 11/1
33

who are treated in a hospital leave it just as quickly as they are able to do so. In the villages of the Punjab I have been able but rarely to obtain a stool specimen from a child, the parents believing that in some occult way I would cast a spell over it.

Some of the charts presented refer to patients treated in the Campbell Hospital at Calcutta and others to natives in the Punjab, cared for by their relatives, and receiving no specific treatment. In both instances the charts are entirely comparable.

Of the 33 patients who have undergone a complete study 12 have died, 21 have recovered. An analysis of the observations made upon this group of cases shows that in 5 cases the virulence of the intestinal bacteriophage for the cholera vibrio was already very high at the time of the first examination, made either at the time of entrance into the hospital at Calcutta or at the first visit in the Punjab. As has been stated above, these initial examinations were made within from 8 to 15 hours after the first appearance of the symptoms. The virulence of the intestinal bacteriophage had then, in these 5 cases, been enhanced very quickly. In all of these 5 patients all morbid symptoms had disappeared 48 hours after the onset and the patients were at this time definitely convalescent. It may be stated here that the infection in these 5 cases was not, at the onset, considered mild; all were considered to be very ill.

Two of the cases are particularly typical. One of them, an Hindoo outcaste, was picked up in a street of Calcutta and carried to the Hospital, which he reached in an absolutely unconscious condition. Collapse was complete and the pulse could not be detected, but the next day all of the symptoms had disappeared and he was anxious to get up. On the morning of the following day, that is, 48 hours after he had been picked up unconscious in the street, the poor man became frightened, and ran out of the hospital. The other case is that of a woman 70 years of age struck down with cholera in a village of the Punjab. As is well known, cholera is, as a rule, fatal in such old people but, as in the preceding case, all of the symptoms had disappeared within 48 hours. I returned to see her the next day and found her attending to her household duties.

In the remaining 16 cases which recovered the intestinal bacteriophage showed but little or no virulence for the cholera vibrio at the time of the first examination, but it became enhanced gradually and 48 hours later was very high. In all of these patients the improvement, and later the disappearance of symptoms, was slower than for the 5 cases of the first group, and a comparative study of the charts clearly shows that the severity of the symptoms at a given time in the disease is directly related to the virulence which the bacteriophage at that time mani-

fests for the cholera vibrio. It may also be noted that the vibrios disappear from the intestines only when the virulence of the bacteriophage reaches a certain limit, a virulence of at least 7 in the scale employed.

In all of the patients whom it has been possible to follow throughout convalescence the virulence of the bacteriophage for the vibrio disappeared shortly after the disappearance of the latter from the intestine. Virulence of the bacteriophage is maintained, then, only so long as the bacteriophage corpuscles are able to develop at the expense of the vibrios.

These observations are still more striking if we examine the charts of those cases which died.

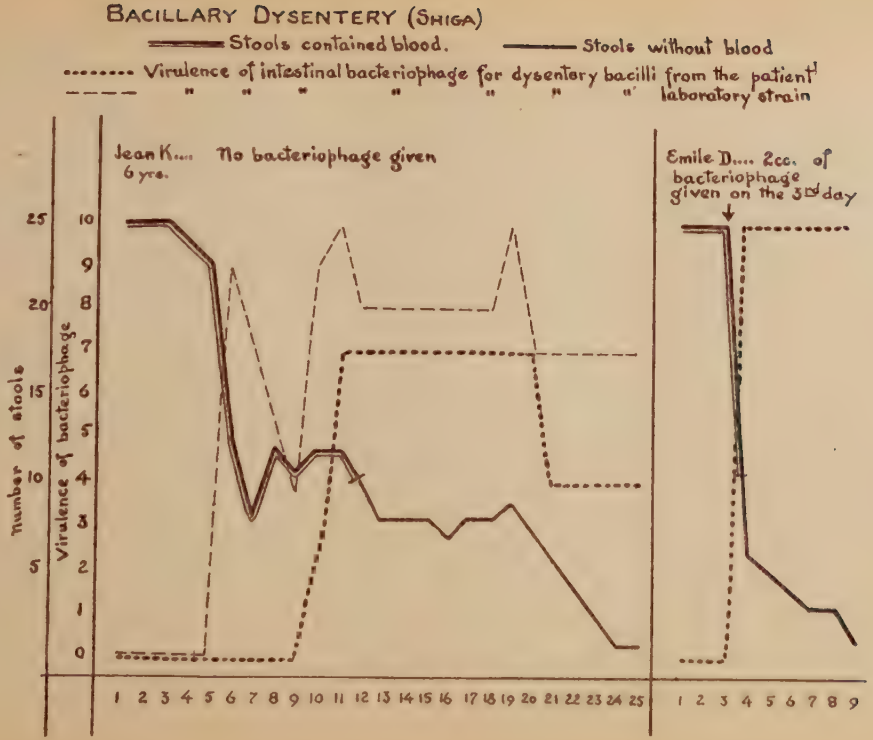
In 9 patients the intestinal bacteriophage showed no virulence for the cholera vibrio at the first examination and it remained inert throughout the course of the disease. All 9 died within 48 hours after the onset of the symptoms.

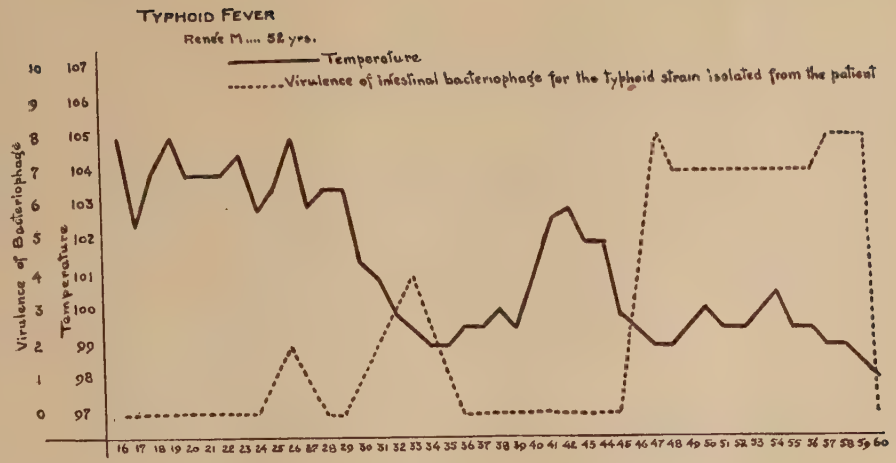
In three patients the bacteriophage showed a weak virulence at the time of the first examination, but this virulence, instead of increasing as in the case of those patients which recovered, became weakened and disappeared. All three died within 24 hours following the disappearance of this virulence. These studies can hardly admit of doubt. It seems clear that the condition of patients affected with cholera is a function of the behavior of the intestinal bacteriophage, and upon it the issue of the disease—death or recovery—depends.

I had previously carried out similar studies on bacillary dysentery, and the conclusions were identical. Recovery depends solely upon the behavior of the bacteriophage, and the condition of the patient faithfully reflects the variations in the struggle taking place within the intestine between the bacteriophage and the pathogenic bacterium. Two charts may here be inserted, as examples, as indicating the results observed in two cases of dysentery. The curve indicating symptoms relates to the number of stools passed each 24 hours. The curve expressing the virulence of the bacteriophage is indicated by the broken line.

It may be well to present also a curve referring to cases of typhoid. The solid line represents the temperature. As may be seen, the conclusions are the same as for the other diseases. This is not a selected case, for in every case studied recovery has followed in an analogous manner. The condition of the patient depends upon the behavior of the bacteriophage and recovery takes place only when the virulence of the bacteriophage reaches an intensity sufficient to lead to bacteriophagy of the pathogenic bacteria.

But, you may say, all diseases are not intestinal. Is the action of the bacteriophage limited to the latter? Typhoid and paratyphoid fevers serve as a connecting link between diseases which are purely intestinal and those which are due to the growth of bacteria in the blood or in the organs.


BACILLARY DYSENTERY (SHIGA)
Stools contained blood.
Stools without blood
Virulence of intestinal bacteriophage for dysentery bacilli from the patient
" " " " " " " laboratory strain
Jean K.... 6 yrs.
No bacteriophage given
Emile D.... 2cc. of bacteriophage given on the 3rd day
Number of stools
Virulence of bacteriophage
25
20
15
10
5
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1 2 3 4 5 6 7 8 9


TYPHOID FEVER
Renée M.... 52 yrs.
Temperature
Virulence of intestinal bacteriophage for the typhoid strain isolated from the patient
Virulence of Bacteriophage
Temperature
0 1 2 3 4 5 6 7 8 9 10
97 98 99 100 101 102 103 104 105 106 107
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

In 1917, I reported that I had isolated a bacteriophage virulent for the paratyphoid bacillus from the urine of a convalescent. This observation indicated that bacteriophage corpuscles do not remain exclusively confined to the intestine, but that they may pass into the circulation. This is by no means surprising, for we know very well that the passage of bacteria from the intestine into the blood is not unusual. The blood of the horse during the digestive period, for example, almost always contains intestinal bacteria, and these bacteria are infinitely larger than is the corpuscle of *Protobios bacteriophagus*.

In discussing an experiment of Bordet, I showed that it is easy to provoke the passage of the bacteriophage from the intestine into the peritoneal cavity. Several authors have since confirmed this, Sumiyoshi among them. By means of a sound he introduced a suspension of bacteriophage into the stomach of guinea-pigs and rabbits. Later he looked for bacteriophage in the peritoneal cavity but failed to find it. When, however, he injected into the peritoneal cavity a culture of bacteria susceptible to the bacteriophage introduced into the stomach a passage of bacteriophage uniformly occurred. In this connection it may be mentioned that Kabelik has shown that the bacteriophage corpuscle exhibits a positive chemotaxis for susceptible bacteria.

Some of my first studies in this connection dealt

with what happens in the experimental disease induced in the white rat following the ingestion of cultures of *B. typhi murium.* I found that when the animal survived, as it frequently did, I could find in the blood, some four to six days after the ingestion, a bacteriophage possessing a virulence for the pathogenic bacterium.

Hauduroy has examined the blood of many patients suffering from typhoid fever and he has regularly isolated a bacteriophage virulent for *B. typhosus* two or three days prior to the fall in temperature, shortly before the disappearance of bacilli from the blood. I may say that it is impossible to find bacteriophage virulent for *B. typhosus* in the blood of either normal individuals or patients affected with other diseases. In other experiments I have found that bacteriophage does not appear in the blood until after it can be detected in the stools. This would indicate that the adaptation takes place within the intestine.

Studies made of an animal disease analogous to human typhoid, avian typhoid, have yielded results comparable in all respects to those secured in human infections, and Kramer, in Holland, has confirmed my results throughout.

In plague also I have found a bacteriophage virulent for the plague bacillus in the stools and in the pus of the buboes at the time of convalescence, as has Avari, in Bombay. The results, as yet unpublished,

obtained by Avari are particularly interesting. The Haffkine Institute of Bombay has carried out studies on plague upon a large scale and has utilized the rat as the experimental animal. But in Bombay, where plague is endemic, between 30 and 99 per cent of the rats are refractory, the percentage varying with the season, while on the contrary, at Madras, a district free of plague, 100 per cent of the rats are susceptible. Avari tried to find a bacteriophage virulent for *B. pestis* in the rats of Bombay but did not find it. These rats thus have a true immunity. In the susceptible rats of Madras the result was the same; a bacteriophage virulent for *B. pestis* could not be found. All of the Madras rats inoculated with plague bacilli contracted the disease, and occasionally a rat, one out of each 200 or 300 inoculated, after having been manifestly sick, recovered. Avari sacrificed these recovered rats and constantly found in the intestine and spleen a bacteriophage possessing a high virulence for *B. pestis*. This he never found in those which died.

These experiments show very clearly that the process of recovery in plague is the same as that in the intestinal diseases. The outcome of the disease depends upon the behavior of the intestinal bacteriophage, whose field of action is not restricted to the intestine. It is capable of passing into the circulation and of inducing bacteriophagy in the fluids and in the organs.

Several authors have isolated from the pus of furuncles and from carbuncles races of bacteriophage virulent for the staphylococcus. I also have studied many cases and I have regularly found it during the suppurative period.

In these diseases we must also consider another very important point. Aside from the direct action of the bacteriophage as a destroyer of bacteria, it exercises a secondary effect. In my first book on Bacteriophage, published in 1921, I described experiments which tend to show that opsonins are in reality the lysins secreted by the bacteriophage corpuscles during bacteriophagy. Let us take two tubes containing like mixtures of sensitive bacteria and leukocytes and let us add to one of these tubes a suspension of bacteriophage or a solution of lysin freed from bacteriophage corpuscles. We will find that phagocytosis is from 5 to 50 times more active in the presence of the lysin than it is in the control tube. The opsonic power of bacteriophage has since been confirmed by various investigators, including Gohs and Jacobsohn, Weiss and Arnold, Nelson, and Smith.

In all diseases where the action of the bacteriophage takes place in the blood stream or in the organs there are, then, two actions, both of which tend toward recovery—the direct action of the bacteriophage in parasitizing bacteria, and the action of the lysins liberated during this process which act as po-

tent opsonins upon the remaining bacteria and, thus lead to their destruction by phagocytosis.*

Which of these two phenomena is the more important? This it is difficult to say at the present time, but this question is of theoretical interest only. From the practical point of view everything depends upon the virulence of the bacteriophage. The higher this virulence the more violent and rapid is the attack and the greater is the quantity of lysin liberated, and at the same time the more vigorous is the opsonic effect. It is, thus, the virulence of the bacteriophage which is the key to the situation in the phenomenon of recovery.

To generalize is hazardous, and naturally we cannot affirm that in all bacterial diseases recovery depends upon the behavior of the intestinal bacteriophage, but the experiments performed thus far justify the statement that in the intestinal diseases of bacterial origin the behavior of the bacteriophage influences the course of the disease, and the issue, death or recovery, depends entirely upon this behavior. In plague and in acute staphylococcus infections, to mention only diseases of man, recovery likewise depends upon the behavior of the bacteriophage. The fact that recovery from such different diseases is under the control

* There is even a third activity: under the action of bacteriophage pathogenic bacteria may lose their virulence. This is particularly striking in the case of the cholera vibrio, for the vibrios of secondary cultures developing *in vitro* or *in vivo* are totally avirulent.

of the bacteriophage warrants the suspicion, at least, that a general phenomenon is operative.

This aspect of the subject cannot be discussed without directing particular attention to one point. Those who may wish to carry out studies of this nature must follow a logical method, that is, they must determine the virulence of the bacteriophage upon each day of the disease from the beginning up to complete convalescence. To determine the virulence on any one day selected by chance is of no value, and it is only necessary to recall the charts presented above to be convinced of this. In the second place, study of the virulence of the bacteriophage must not be limited to determining the action of the filtrate upon a single culture of the organism selected by chance. In each case it is essential to isolate the pathogenic bacterium from the patient himself at the beginning of the disease and it is with cultures of this bacterium that the different filtrates derived from the specimens obtained upon each day of the disease must be tested for bacteriophagic action.

Needless to say, such a field of study is open to all bacteriologists, and yet it is necessary that these bacteriologists be trained in the manipulation of bacteriophage. And finally, let me repeat that it is essential to study *natural* disease. Upon several occasions I have stated that experimental diseases are mere artificial phenomena, leading always to conclusions

equally artificial. There is no need to emphasize this point further.

Returning to the subject under discussion, it is obvious that the study of the pathogenesis of infectious disease indicates that the phenomenon of recovery depends upon the behavior of the intestinal bacteriophage, and if this deduction is true, this conclusion is susceptible to proof by means of a "cross experiment." It is possible, indeed it is easy, to cultivate races of bacteriophage of enhanced virulence. It is only necessary to introduce into a culture of the pathogenic bacterium a trace of a suspension of a virulent bacteriophage; the bacteria are attacked and destroyed, and meanwhile the bacteriophage multiplies. That which was at the beginning a culture of bacteria becomes, after a few hours, a culture of bacteriophage.

Let us give a patient by mouth, at the onset of symptoms, a few drops of this culture of bacteriophage. Bacteriophagy will take place *in vivo* and recovery will follow. The patient will not be forced to take the chance of his own intestinal bacteriophage undergoing an adaptation, for we can inaugurate, at the beginning of the disease, the natural processes of recovery.

From 1919 on, I have made experiments upon patients affected with bacillary dysentery, causing each patient to ingest 2 cc. of a culture of bacteriophage

having a high virulence for dysentery bacilli. In all cases, without exception, all of the morbid symptoms disappeared within a few hours, in from 4 to 20 according to the case, and the next day the patient was definitely convalescent. Since that time, this method of treatment has been applied on a large scale, principally in the Soudan and in Brazil.

In Brazil, as the result of control experiments conducted by da Costa Cruz, who obtained results identical with those which I had reported, the Oswaldo Cruz Institute of the Brazilian government has prepared, since 1924, cultures of a bacteriophage highly virulent for dysentery bacilli. These have been placed in 2 cc. ampoules and distributed to hospitals, to government health officers, and to all physicians who have requested them. This mode of treatment has quickly supplanted all others, including the use of antidysenteric serum, which has been abandoned. The results obtained in the first 10,000 cases have been published and only two failures are recorded.

As for the Soudan, the results may be summarized by the following statement, made by the Director of the Medical Service: "The results of treatment of bacillary dysentery with it have been little short of miraculous." A single failure, the case of an infant already moribund when brought into the hospital, occurred among several hundred cases treated.

An experiment of this kind, having been carried

out on some tens of thousands of cases, justifies the statement that bacteriophage treatment constitutes the specific therapy of acute bacillary dysentery, and at the same time it proves that natural recovery from dysentery is effected through the same agent.

I will only mention here that success has attended the treatment of staphylococcus and of *B. coli* infections. The routine therapy of these infections has been applied most extensively in the hospitals of Paris. Recently several papers have been published on this subject in the United States and they indicate the great value of bacteriophage treatment.

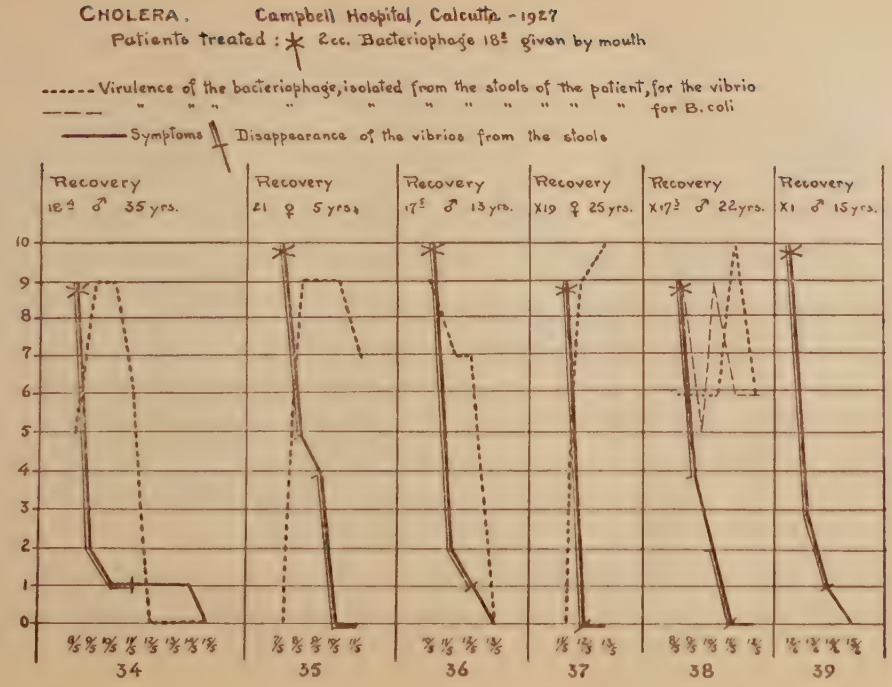
During the past year, while in India, as the result of the experiments of which I have spoken, I attempted the treatment of Asiatic cholera. These attempts at therapy were made in the Punjab, on natives cared for in their homes and to whom no other medication was given. Each patient received an initial dose of 2 cc. of a virulent bacteriophage, and with the family a second dose of 4 cc. diluted in 100 cc. of water was left, with instructions to give it to the patient by the spoonful during the 3 or 4 hours following. I should state that I merely furnished the cultures of bacteriophage; treatment was carried out by Major Malone of the Indian Medical Service, assisted by the other officers of the Service. As it was impossible to enforce any one mode of treatment, the family of the patient was free to accept or refuse it,

in the latter case usually resorting to the prescriptions of the Hindoo physician. The majority of the patients for whom authorization was granted were found in a critical state, indeed, it was only because of this that parents, despairing of saving them, accepted the new treatment. The controls, by means of which the value of the treatment could be ascertained, were provided by those cases for which bacteriophage treatment was refused. In spite of these extremely unfavorable conditions the mortality in the controls was 62.9 per cent, and among those treated with bacteriophage 8.1 per cent. Similar results have been recently reported by Colonel Morison of the Indian Medical Service, who has treated cases in Assam.

As the result of these experiments the Indian Government has created a special laboratory for the preparation of bacteriophage cultures on a large scale, with a view to the treatment of cholera and dysentery. This laboratory will also carry out studies to the end of extending this mode of treatment to other diseases, to plague in particular, with which I have already obtained some interesting results.

To cure disease once it has developed is not without interest, but to prevent the disease is still more important.

Since bacteriophage abounds in the intestinal contents, the convalescent must distribute in his excreta


CHOLERA. Campbell Hospital, Calcutta - 1927
Patients treated : * 2cc. Bacteriophage 18/2 given by mouth
------ Virulence of the bacteriophage, isolated from the stools of the patient, for the vibrio
— — — " " " " " " " " " " " for B. coli
——— Symptoms ‡ Disappearance of the vibrios from the stools
Recovery
18/2 ♂ 35 yrs.
Recovery
21 ♀ 5 yrs.
Recovery
17/5 ♂ 13 yrs.
Recovery
X19 ♀ 25 yrs.
Recovery
X17/3 ♂ 22 yrs.
Recovery
X1 ♂ 15 yrs.
10
9
8
7
6
5
4
3
2
1
0
34
35
36
37
38
39

those races of bacteriophage which have undergone adaptation within him, and which, consequently, possess a high virulence for the pathogenic bacterium causing the epidemic. Pathogenic bacteria become scattered throughout the environment of the patient and the enhanced bacteriophage is distributed by the convalescent. The first of these propagate the disease; the second should propagate recovery.

In 1918, I had the opportunity to make some interesting observations during an epidemic of dysentery then present in the neighborhood of Paris. I isolated a race of bacteriophage very virulent for dysentery and paradysentery bacilli from several healthy persons who had been in contact with patients. I also examined the stools of individuals affected with a transitory, simple diarrhea and in each case I demonstrated bacteriophage virulent for dysentery bacilli. From these facts I concluded that during an epidemic, pathogenic bacteria must become disseminated so widely that but few individuals remain free of contamination, but that to offset this, the opportunities for "contamination" by an adapted bacteriophage derived from convalescents must be even greater. Apparently everyone who became "contaminated" by such a bacteriophage became thereby refractory and if later they ingested dysentery bacilli the latter must be immediately destroyed by bacteriophagy.

However, a single "contamination" by the en-

hanced bacteriophage could not be sufficient to render a person permanently free of contagion, for earlier experiments had shown that when a suspension of bacteriophage was ingested, elimination was complete within three or four days. The normal bacteriophage which grows in the intestinal tract of all persons is permanent simply because it lives in symbiosis with *B. coli* and the ingested foreign bacteriophage must be eliminated unless it encounters, within the body, bacteria at the expense of which it can develop. It must be necessary, then, if an individual is to be continually refractory, that he be subject to frequent "contamination" by the adapted bacteriophage.

This hypothesis, suggested by these facts, induced me to extend these studies. Within a short time I had an opportunity to study several epizoötics of fowl-typhoid, a disease of *Gallinae* closely resembling typhoid fever, and the observations there made demonstrated the following points:

1. Outside of districts where an epizoötic of fowl-typhoid was present the intestinal tract of chickens never contains a bacteriophage virulent for *B. gallinarum,* the agent of the disease.

2. Recovery in fowl-typhoid is rare (at least it was in the epizoötics which I studied), but when it does occur a bacteriophage extremely virulent for the pathogenic bacterium is present in the intestine.

3. An abrupt cessation of the epizoötic is usually

coincident with the recovery of a chicken and, although prior to this recovery none of the chickens reveal a bacteriophage highly virulent for *B. gallinarum,* upon the day following the recovery the enhanced bacteriophage is to be found in the excreta of all chickens living in the barnyard. The bacteriophage of enhanced virulence must, therefore, be derived from the recovered chicken which disseminates it with its excreta. This race of bacteriophage is then ingested by the other chickens which thenceforth become free of pathogenic bacteria, for the latter are bacteriophaged just as soon as they enter the digestive tract.

While in Indo-China in 1920, I carried out extensive studies on another disease—hemorrhagic septicemia of the water-buffalo. Outside of regions where the disease was present I never found within the intestinal contents of the buffalo, races of bacteriophage virulent for the Pasteurella, the cause of the septicemia. I observed an epizoötic in which more than 10,000 buffaloes died, and I examined the intestinal contents of many cadavers, but found no races of bacteriophage virulent for the Pasteurella. And yet, at the end of the epizoötic a bacteriophage highly virulent for this Pasteurella was present in the excreta of all buffaloes which had resisted contagion. Here again but a single explanation is possible. The occasional animal which recovers disseminates the en-

hanced bacteriophage and the water-buffaloes which ingest it are thus protected from the disease.

In 1927, while in India, I studied the epidemiology of cholera and observed a repetition of the same facts. The bacteria of the disease are imported into a town or village by a person actually suffering from the disease, or by one who is in the incubation period. From this person the cholera vibrios are distributed throughout the environment and the epidemic begins. The first cases are usually fatal. Then a patient recovers, through the mechanism already discussed. From this convalescent, bacteriophage adapted to the destruction of the cholera vibrio is spread throughout the environment, and the recoveries become more and more frequent, the epidemic finally ending when the bacteriophage adapted to the cholera vibrio has become widely disseminated.

The course of an epidemic reflects, then, a struggle between the pathogenic vibrio and its parasite, the bacteriophage. And, if this is true, logically it should be possible to stop an epidemic at its beginning by the simple expedient of distributing cultures of virulent bacteriophage in such a way that the contamination would quickly become general. In a word, it should be possible to duplicate experimentally the natural conditions which bring an epidemic to a close.

This is what I attempted to do with cholera in the villages of the Punjab. At the appearance of an epi-

demic I poured into each of the wells of these villages two ounces of virulent bacteriophage culture. In all instances the epidemic suddenly stopped. From the next day on, no new cases of cholera developed. As examples I mention the two following instances.

Ghang is a village of 1,500 inhabitants. Cholera broke out on August 16 and by the 18th 22 cases had developed. At this time I poured the bacteriophage cultures into the wells providing the potable water. The epidemic considerably diminished but six additional cases occurred between the 18th and the 25th of August. At this time an inquiry was conducted by the officers of the Indian Medical Service and revealed the fact that the inhabitants had concealed a private well located in the courtyard of a house close to the village. This well had remained untreated and had been used by the friends of the owner. It was then treated with bacteriophage and from then on no further cases of cholera developed.

Nawar is a village of 2,000 inhabitants. Cholera broke out on August 2. Six inhabitants contracted the disease within a period of a few hours' time and all died during the following night. The population was alarmed and announced that it was ready to adopt any measures that might be proposed. On the morning of August 3, at the time of our visit, there were six new cases which had developed during the night. The two wells providing the potable water

were treated with bacteriophage, and the patients, as well as the inhabitants who were still well, drank this water. The six patients recovered and no new cases developed.

In all of the experiments carried out the results were similar.

After all of these experiments I believe that no doubt can be entertained. In all of the diseases which we have considered, the aspect of the disease in the individual depends upon two factors operating in opposite directions. On the one hand, the bacterium, and on the other, the bacteriophage, a parasite of the bacterium, and it is the behavior of the bacteriophage which determines the issue. It is the degree of dissemination of bacteria on one hand and of bacteriophage on the other which regulates the progress of an epidemic; a general diffusion of virulent bacteriophage marks the end.

A disease is contagious because of the bacteria which spread it; recovery is contagious because of the bacteriophage which is disseminated. We are able at will to reproduce the natural processes of recovery, just as we are able to reproduce experimentally the natural processes leading to the disappearance of the epidemic.

Are these phenomena limited to the diseases studied up to the present time? Only future studies can answer.

CHAPTER VI

The Use of Bacteriophage

THE data presented in the previous chapters, in particular those bearing upon the subjects of bacillary dysentery and cholera, make it obvious that in bacteriophage we have an agency which should readily lend itself to therapeutic study and, indeed, such studies have already been made in many infectious diseases of animals and man.

The first of the diseases of man to be given serious consideration from the standpoint of bacteriophage therapy was bacillary dysentery, and the results of these initial studies were first published in 1921, in the French edition of The Bacteriophage, Its Rôle in Immunity. Since that time much additional work has been done in connection with this disease, and, aside from but a few isolated reports, the results have been but a confirmation of the statements there made. In a few instances, however, investigators have reported that in their attempts at the treatment of the bacillary dysenteries the results were unqualified failures and, indeed, in most cases certainly, failure might have been anticipated since the conditions governing the work were insufficiently controlled.

Many authors attempted to repeat my early experiments on the therapeutic use of the bacteriophage without first repeating my studies of bacteriophagy *in vitro*. Only such preliminary experiments would have informed them as to the basic behavior of the bacteriophage and impressed upon them the fact of greatest moment that each race of bacteriophage possesses its own distinctive virulence. At the time of the first publication on the treatment of bacillary dysentery emphasis was placed upon the fact that only very virulent races of bacteriophage are competent to cause complete bacteriophagy *in vivo*, and that those who sought to utilize strains of low virulence could not expect results. Unfortunately, many authors have paid no attention to this recommendation, doubtless relying upon the false belief, then quite generally held, that the bacteriophage was a ferment. And how, indeed, can a ferment possess a variable virulence? This erroneous conception as to the nature of bacteriophage naturally led to the belief that all races of bacteriophage were identical. Consequently, they gave their patients the first race at hand with no concern as to its virulence. From the failures experienced they concluded that the method of therapy was without value, when, as a matter of fact, their lack of success was determined solely by their inadequate technic.

That this explanation is true is clearly shown by the

results reported at various times by da Costa Cruz. In 1922, he reported that he was unable to confirm my studies and concluded that in the treatment of bacillary dysentery bacteriophage exerted no effect, but after having continued his studies of the properties of bacteriophage, he repeated in 1923, his clinical experiments and reported a complete success. In this last work he had paid due regard to the importance of virulence and to the differences in virulence exhibited by different races of bacteriophage. The result has been that at the present time bacteriophage treatment of the bacillary dysenteries is the only method of therapy employed in Brazil.

In this connection too much emphasis cannot be placed upon the fact that bacteriophage acts effectively only when very virulent races are employed. The effect of bacteriophage is not proportionate to the virulence; races of low virulence do not exert a slight effect, preparations of moderate virulence a somewhat greater effect, and highly potent races a profound effect. Bacteriophage is either therapeutically effective or it is without benefit. As a matter of fact the use of preparations of low virulence may be of distinct disadvantage to the infected host, since such races may give rise to the development of resistant bacterial strains within the body.

Another aspect of the problem, of considerable moment, is the question of the mode of bacteriophage

administration. This has not, in all instances, received adequate consideration; acute and chronic infections, localized and more general processes have all been treated in much the same fashion. This can hardly be logical, since from the point of view of the three beings involved—man, bacterium, and bacteriophage—the interrelationships in the acute infectious process must be quite different from the conditions in the chronic process. It is readily apparent that the acute state not infrequently becomes the chronic condition, and let me at once answer an objection which you may bring up. Quite logically you may ask why, if the bacteriophage naturally adapts itself to the destruction of pathogenic bacteria, there are chronic infectious diseases. Why does not the natural process of recovery uniformly intervene? With a potent race of bacteriophage undergoing prompt adaptation recovery follows; with a race of very low virulence incapable of adaptation, bacterial activity is unimpeded and the host dies. What is the intermediate condition, and how is it brought about? The answer is obvious. All of the beings involved are biological systems. Bacteria are living beings and, as such, they possess the power of adapting themselves to adverse conditions. When attacked by a powerful bacteriophage, they succumb, but if the destructive power of the bacteriophage is weak, the bacteria resist and acquire a real immunity to the bacteriophage. At the

same time profound modifications occur in the character of the bacteria, the main change being, as far as the present subject is concerned, an attenuation in the virulence of the germs.[1]

Chronic diseases are caused by bacteria in which a state of symbiosis has been established between the bacterium and the bacteriophage, and in this symbiotic relationship the bacteria naturally resist the action of the bacteriophage. Nevertheless, under such conditions a therapeutic effect through bacteriophage action is still possible, provided a race is employed whose virulence exceeds that of the bacteriophage

[1] This attenuation of virulence is usually an accompaniment of an increased toxigenic property. Early in my studies on this subject, I inoculated animals with secondary cultures and, observing that these animals died more quickly than did the controls which had received normal cultures, I concluded that the *virulence* of the bacteria of the secondary cultures was increased. I have since seen that this conclusion is erroneous, and I very frankly admit that of all faults it is the one of which I should not have been guilty, for I have often protested against the errors incidental to experimentation upon *refractory* animals. As a matter of fact, neither the normal control cultures nor the secondary cultures under test were *virulent* for the experimental animals: their death was due to the *toxicity* of the inoculated culture, and if the animals died more promptly after inoculation of the secondary cultures it was only because they were more toxic than were the normal cultures. The same situation is encountered in the case of the cholera vibrio. Secondary cultures of the vibrio are completely avirulent for man, the only naturally susceptible animal. Nevertheless, by the injection of guinea pigs or rabbits (refractory species) with normal cultures or with secondary cultures, it is found that the minimal lethal dose of the secondary culture is much smaller than is that of the normal strain, indicating, of course, a greater toxicity.

with which the bacterium is already in a state of symbiosis.

There is an essential difference in principle between the treatment of acute and chronic infectious diseases by means of the bacteriophage. In the case of acute diseases, powerful bacteriophage must be brought into contact with the pathogenic bacteria to destroy them before they have had time to occasion lesions sufficient to cause death. Bacteriophage therapy should be instituted as early in the infection as possible. In the case of chronic diseases, the patient must receive a bacteriophage having a destructive power so great that the already resisting bacteria are destroyed. In the latter case, the uncertainty of the treatment is evidently greater than is the case in acute infections.

In acute diseases, it is sufficient to apply, *as soon as the first symptoms are noticed,* or early during the course of the disease, a small quantity of a potent "stock" bacteriophage in order to occasion the destruction of the bacteria and thus bring about recovery. In chronic diseases, a resistance, more or less marked, must be overcome. A powerful "stock" bacteriophage is often sufficient to accomplish this, but in some cases it is essential to make use of what may be termed an "auto-bacteriophage", that is to say, a bacteriophage trained beforehand to destroy *in vitro* the resisting pathogenic bacteria obtained from the patient himself.

Even this procedure does not solve all of the problems attending therapy, for it must be frankly admitted that there are chronic cases in which bacteriophage treatment remains entirely powerless. With further perfection of our technic for development of more potent races of bacteriophage it may be possible in time to treat successfully these more resistant cases also.

In considering the use of bacteriophage in therapy it should be pointed out that the treatment of disease by means of bacteriophage suspension in reality brings into action a complex of three different mechanisms each of which plays a rôle in combating the infection and in determining the refractory state following recovery.

First, and perhaps of greatest immediate importance is the direct action of the bacteriophage, leading to a more or less complete dissolution of the invading bacteria. Some investigators deny that the most important feature of bacteriophage therapy is the dissolution of the bacteria responsible for the infection and they attribute the therapeutic results primarily to the effect of the dissolved bacterial proteins. I should like to call attention to the fact that these investigators stress the importance of using highly potent polyvalent races of bacteriophage. This fact in itself indicates that the thing of primary importance is the actual dissolution of the invading bacteria. This is further emphasized by the fact that when one ad-

ministers a bacteriophage which is incapable of dissolving the microorganism responsible for the particular infection, of the urinary tract, for example, no therapeutic results are realized. This is a proof that actual lysis is essential in effecting a cure and that in the absence of real dissolution the rôle played by the bacterial proteins themselves is nil.

A second mode of action, as I demonstrated years ago, and this has since been confirmed by a number of American investigators, depends upon the fact that suspensions of bacteriophage act in a manner identical with potent opsonins. I have been able to show that this effect is exerted by the lysins secreted by the bacteriophage corpuscles during the process of bacterial dissolution. This enhancement of phagocytic action may be by no means unimportant.

It may be well to answer here an objection which has been raised, namely, that since *in vitro* secondary cultures may be produced, even with potent races of bacteriophage, such cultures must also be produced *in vivo.* Those who have formulated this objection assume, *a priori,* that the action *in vivo* is identical with that *in vitro.* But this is not true. The opsonic action of the bacteriophage, which is beyond question, obviously can take place only within the body, and the combined intervention of the two destructive actions renders, *in vivo,* the activity of the bacteriophage not only more rapid but also more complete.

It is of further significance that the opsonic action of the lysins is, as experiment has shown, not wholly specific, and is of such a nature as to explain certain of the "non-specific" therapeutic results to be discussed in the following pages.

A third mechanism which also plays an important rôle, particularly in the more permanent resistance which the host may develop against the specific microorganism, is referable to the fact that during the process of dissolution, the bacterial proteins are degraded to a particular state which possesses the power of inducing in the host a refractory state of more or less duration. In other words, the bacterial split-products obtained under the action of the bacteriophage are in a physical or chemical state highly suitable to induce a strong and durable immunity. The extensive studies made upon the vaccination of buffaloes against hemorrhagic septicemia in Indo-China, in 1920, were described in The Bacteriophage, Its Rôle in Immunity, and justify this conclusion. In those experiments I observed, after the injection of a minute dose of a bacteriophage suspension, that the buffaloes exhibited a refractory state of very short duration (two or three days). This was due to the presence of the bacteriophage itself. Then, following this immunity, there was a period during which the animal again became susceptible, the length of which being in inverse proportion to the quantity of bacteriophage

suspension injected (about 18 days for an injection of 0.5 to 1 cc., more than 45 days for an injection of 20 cc.). This period of susceptibility was followed by a second refractory period of long duration (more than a year) which cannot be attributed to the presence of bacteriophage and can only be explained as due to the immunizing action of the bacterial proteins contained in the suspension injected.

The rôle of the bacteriophage is, therefore, complex, but the principal therapeutic action is due to the direct action of the bacteriophage itself, as we shall see later.

To return more specifically to the question of bacteriophage therapy, it is clear that bacteriophage treatment does not constitute an exception among therapeutic procedures. It has long been known that in serological therapy the effectiveness of treatment is directly related to the time at which the serum is given. In bacteriophage therapy likewise, the assurance of satisfactory results is the greater when bacteriophage is administered early in the disease. In certain respects an almost complete analogy obtains between bacteriophage therapy and serum therapy; once lesions have developed neither serum therapy nor bacteriophage therapy is competent to restore, or, through direct action, to heal these lesions. Antitoxin merely neutralizes toxin and in that way prevents the continued development of the lesions; bacteriophage acts

upon the infecting bacteria and thus interrupts their continued development with the progressive extension of the lesion.

The mode of application in bacteriophage therapy is extremely simple. Obviously, if bacteriophage is to be operative upon the infecting organisms it must come into direct contact with them, and a method of administration must be selected which will effect this end. For instance, in intestinal disorders the bacteriophage should be given by mouth, since by this route it can most readily come into contact with the bacteria proliferating in the intestinal tract.

In cases of infection of the genito-urinary tract, such as cystitis, the bacteriophage should be introduced directly into the bladder or even directly into the pelvis of the kidney in cases where kidney lesions are present. Finally, in the case of localized infections of cutaneous or subcutaneous tissues, the bacteriophage should be introduced directly in the infected focus.

From the standpoint of dosage and administration two factors must be considered:—the quantity of bacteriophage to be given, and the frequency and number of treatments.

With regard to the first of these, that is, the quantity of bacteriophage introduced, it must be stated that this is of less importance in the case of bacteriophage therapy than in most other therapeutic procedures. The important consideration is the virulence of the

bacteriophage, rather than the amount. This is true, because, as has been explained previously, bacteriophage in the presence of susceptible organisms perpetuates itself and the amount administered does not determine the amount of bacteriophage ultimately to develop.

The number of administrations essential to induce a therapeutic effect varies with the type of condition under treatment; in acute infections or in infectious conditions that have not become fully chronic, a single administration or, perhaps, two administrations should overcome the organisms. In chronic conditions, on the other hand, it may be necessary to continue bacteriophage therapy over a relatively long period.

With regard to the frequency of treatments, this, again, must vary with the disease under consideration.

Bacteriophage preparations designed for therapeutic purposes are prepared in exactly the same way as is bacteriophage for experimental study. In Chapter I the method is described for preparing and developing highly virulent races of bacteriophage. That method in all of its essential details is the method to be employed in producing a virulent bacteriophage for use in treating disease. It is essential that the culture, after dissolution, be subjected to filtration in order that organisms of secondary growth may with certainty be eliminated. It is further desirable that

the filtrate so obtained be subjected to a period of incubation prior to its use. This will not only guarantee against contaminations that might be associated with manipulation but insures against the presence of organisms in the filtrate, organisms which might develop from filter-passing bacterial forms.

Along with the factor of virulence the element of specificity enters into the problem of therapy. It is essential to bear in mind the distinctive behavior of certain bacterial species. Within some species individual strains or cultures possess attributes which determine their behavior with reference to bacteriophage, while in another species behavior is governed by characteristics which are species specific rather than strain specific. Thus, in the absence of an acquired resistance to bacteriophage, all strains of *B. dysenteriae* Shiga undergo bacteriophagy with *any* race of bacteriophage active for any culture of this organism. Such a species is homogeneous. With *B. coli* and with the staphylococcus, for example, such uniformity of behavior does not obtain, for a given strain of the staphylococcus may be highly sensitive to one race of bacteriophage and completely refractory to another, even though the latter is highly active with another culture of the staphylococcus. Such bacterial species are heterogenic; in them strain specificity transcends the attributes referable to species. It should not be inferred that the element of specificity mentioned here

necessarily implies that in all infections in which the organism belongs to a heterogenic species but a single race of bacteriophage is effective. In the case of races active against such organisms as the staphylococcus or *B. coli,* certain races of bacteriophage may exhibit a considerable breadth of action, being effective against diverse bacterial strains.

The question of virulence has been mentioned and emphasis placed upon the necessity of utilizing a race of maximum virulence. By this is meant a race of bacteriophage which will cause a complete and permanent dissolution of the organisms *actually present* in the infectious process.

Bearing in mind, therefore, that in bacteriophage therapy as in therapeutic procedures of other types, success can be anticipated only when the nature of the reaction is understood and conditions are so controlled that a maximum effect may take place, we may now consider the practical application of bacteriophage therapy and present in more detail the methods used and the results obtained in the various diseases which have, up to the present time, been treated by this method.

Infections Due to Bacilli of the Enteric Group

Bacillary Dysentery

The relationship of bacteriophage to this disease, including its therapeutic application, has already been

considered. Nothing would be gained by repeating in detail the statements already made, but it may be said that treatment of bacillary dysentery by means of bacteriophage has become a standard procedure. It has been carried out over such a period of time that it is unnecessary, indeed, quite impossible to recall all of the work reported. It may be of interest, however, to mention one recent report, in which the treatments were carried out under conditions that made the work most difficult, and in view of the results obtained, conclusively show the effectiveness of bacteriophage therapy. This series of cases, reported by Choudury and Morison of the Pasteur Institute at Shillong, deals with an outbreak of dysentery in the native villages in the jungle, and the results were so clear-cut that it is impossible to do better than to summarize them. It may be pertinent to point out that this mode of treatment is in itself so simple and so free from danger that it can be applied under most unfavorable conditions and, indeed, even by the natives themselves.

In this epidemic there were but 70 cases treated, of which 43 were classified as moderately severe and 18 as severe. The sole treatment consisted in administering 2 cc. of bacteriophage by mouth three times during the first day, with later treatments, where indicated, by the same route. Of the 70 cases but three died and these were in the group classified as extremely ill at the time treatment was instituted. Two

of the fatal cases were infants. All except one of the patients recovered had resumed work within 14 days after treatment was instituted. Certainly results of this type are not open to question and, taken in conjunction with the thousands of cases successfully treated elsewhere, leave no doubt as to the efficacy of bacteriophage therapy in bacillary dysentery. It should be pointed out that in discussing dysentery reference is made to bacillary dysentery only; in amebic dysentery bacteriophage is entirely without effect.

Cholera

The value of bacteriophage therapy in this disease has also been discussed in the previous chapter. Further comment would appear to be unnecessary but it may be permissible to mention a recent report from Assam, where the treatments were carried out under conditions as unfavorable as were those mentioned in connection with dysentery. Briefly, the results reported were: Cases receiving no bacteriophage, 29, deaths, 22; cases receiving bacteriophage, 31, deaths, 9. As is obvious, these results are entirely in accord with those mentioned in the preceding chapter. It may be mentioned that the results just reported are based upon an epidemic of cholera in which the mortality was exceptionally high and it is also worthy of note that among the treated cases there were four individuals who were moribund at the time treat-

ment was instituted and, as has been said, in cases where the lesions of the disease have become sufficiently extensive bacteriophage therapy can be of no avail.

Diarrhea in Children

The treatment of the infantile diarrheas by bacteriophage has not been as uniformly satisfactory as has been the treatment of dysentery and cholera. In many instances the reasons for this are obvious. As has been mentioned above, the questions of specificity and potency have not received adequate attention and, undoubtedly, many of the failures are directly referable to such causes. But however this may be, it is equally true that in studies made in 1919, under conditions where the importance of these factors was recognized, and in various other studies made since that time, the results have shown, that given satisfactory conditions, bacteriophage therapy is effective in diarrheal disturbances of this type. These studies indicate that when we have obtained a thorough knowledge with regard to the causative agents associated with such conditions and have developed a bacteriophage sufficiently polyvalent to act upon the organisms involved, successful treatment may more uniformly be expected. Despite the fact that at the present time this information is not available it should be pointed out that there is no reason why bacteriophage therapy should not be instituted with the materials now available. If the bac-

teriophage is effective, well and good, improvement is prompt; if it is not effective it can do no possible harm and can interfere in no way with any other mode of therapy that might be applied. Analysis of the reports thus far made shows that the treatment is attended by success in about 60 per cent of the cases.

As an example, the following case, taken from a group of 18 cases similarly treated, may be mentioned.

July 7, 1928, K. . . . . 7 months of age.

For three days the child had exhibited a profuse diarrhea. The abdomen was markedly distended. The gastro-intestinal disturbance was marked and the child could not retain water. The parents of the child exhibited no abnormal symptoms. On the day of observation the child received 2 cc. of bacteriophage diluted in 40 cc. of water. One spoonful of this was given by mouth every hour and during the day the child received two enemas of bacteriophage in the same dilution. On the following day the enemas were repeated. After 48 hours the stools became normal and the child again began nursing. Recovery was complete.

Results of this type, when contrasted with the failures reported, indicate beyond question that with added knowledge of the etiology of these diarrheal conditions, together with the isolation of suitable potent races of bacteriophage and the development of

effective modes of application, much may be expected from this method of treatment. Our knowledge of the mechanism of spontaneous recovery from infections of this type but confirms this conclusion.

Colitis

Despite the fact that from the etiological standpoint disturbances of this type are not thoroughly understood, it is true that in many instances where such conditions have been present, bacteriophage therapy has been applied with beneficial results. Da Costa Cruz has used antidysentery bacteriophage, and I have treated cases of this type with mixtures of bacteriophage active upon various organisms of the enteric group. Successful results have been recorded. The explanation for the effectiveness of such preparations is not entirely clear. Two possibilities suggest themselves, each of which may be of considerable significance. First, as has been described above, many bacteriophage races exhibit a considerable range of virulences and the races employed by da Costa Cruz, for example, may well have been active for organisms, possibly of the paradysentery types, associated with the disturbance. The second possibility has already been discussed in detail, when it was shown that bacteriophage may more or less readily adapt itself to a new bacterial host. It may well be that in this adaptation we have an explanation for the

mode of action of bacteriophage in the treatment of disturbances of this nature.

In enterocolitis and disturbances of like nature, the percentage of those recovering following bacteriophage therapy is lower than in many other types of infection. About 40 per cent of the cases so treated show evident beneficial effects of the treatment.

The following case may be cited as an example of the mode of action.

On Jan. 3, 1928, Mrs. M., 37 years of age, presented the following history:

The patient complained of pain in the right iliac region. There was marked emaciation, and the complexion was sallow. There was mental depression and irritability. Frequently there had been a complete intolerance to food, lasting over a period of two or three days. Periods of obstinate constipation were interrupted by diarrheal stools associated with pronounced intestinal putrefaction. Mucoid stools indicated inflammation of the colon. Only a very selected diet could be tolerated. This condition had been present for some time and four years previously the patient had undergone an appendectomy without beneficial effect. The patient is married but has no children. On the day of admission, bacteriophage treatment per os was instituted, 2 cc. of bacteriophage being given in the morning and again at night. On January 9, the stools had become firmer and of normal

appearance, and had almost completely lost their characteristic odor. The general health was much improved. Throughout a period of three weeks treatment was continued and the patient gained two kilograms in weight and was able to take a normal diet without appreciable ill effects. This patient was examined periodically until August, being given bacteriophage treatment every two months. On September 3, the general condition was markedly improved, stools were formed and bowel movements were regular. She had gained a total of 4.5 kilograms.

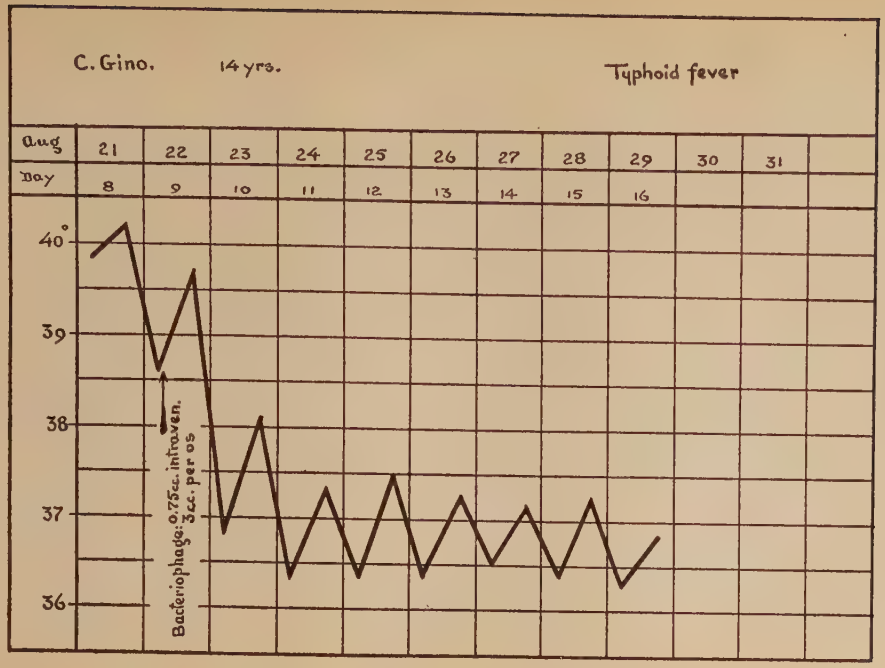
Bacteriophage therapy obviously offers possibilities in cases of this type. As in the case of the infantile diarrheas the situation here is complicated by the question of etiology. Naturally, bacteriophage treatment can be expected to yield results only in those cases of infectious origin, and what organism, or how many organisms, may be involved as incitants is not yet entirely clear. It may well be that a more intensive bacteriologic study of enterocolitis will point the way to a more effective bacteriophage therapy.

Typhoid and Paratyphoid Fevers

Contrary to what might be anticipated in view of the effectiveness of bacteriophage therapy in other infections of the intestinal tract, such as cholera and dysentery, the use of bacteriophage in the typhoid and paratyphoid infections of man has not been at-

tended by any considerable success, although certain authors, notably Alessandrini and Doria, in Italy, have reported favorable results. As a general rule most investigators, among whom may be mentioned Wolff in particular, have failed to find any evidence of therapeutic efficiency, and during the past year, in connection with an outbreak of typhoid fever in Lyons, studies made under my direction have given entirely negative results. This fact is the more surprising, particularly in connection with the Lyons outbreak, when it is borne in mind that in the cases there treated the bacteriophage used was actually known by laboratory control to be virulent for the infecting organism. This but serves to emphasize the fact that our knowledge with regard to infections of this type is far from complete. Manifestly, in view of these results and of the diverse reports that have been published, it can only be said that at the present time the question of the application of the bacteriophage to typhoid fever remains unsettled. This is, perhaps, the more disturbing in view of the occasional favorable reports that have appeared. As an example of these, a case of Alessandrini and Doria may be cited.

C. Gino, age 14 years, developed, on the 12th of August, a fever with the temperature varying between 38 and 40.5°C. Rose spots developed and the spleen became palpable. The blood culture was positive for *B. typhosus*. The Widal was positive in a dilution of


C. Gino. 14 yrs.
Typhoid fever
Aug
Day
21 22 23 24 25 26 27 28 29 30 31
8 9 10 11 12 13 14 15 16
40°
39
38
37
36
Bacteriophage: 0.75cc. intraven.
3cc. per os

1:600. The typhoid bacillus strain isolated from the patient proved to be susceptible to bacteriophage. On August 22, at 10 A.M., 0.75 cc. of typhoid bacteriophage was injected intravenously and 3 cc. were given by mouth. After a period of about 20 minutes the temperature rose suddenly to 39.8°C., with cyanosis and other evidences of an intense reaction. By noon this reaction had largely disappeared, and on the 23rd of August the temperature fell to 37.4°C. and upon the following day it fell still lower, where it remained until recovery was complete on August 28.

This case shows that, given suitable conditions, bacteriophage is effective here as in other diseases, but as yet the essential conditions are not understood and are beyond control.

Colon Bacillus Infections

The colon bacillus is probably the most heterogenic of all bacterial species and it is to this fact, perhaps, that some of the disagreement in the results dealing with the use of bacteriophage may be due. In view of this distinct strain behavior, in applying bacteriophage therapy in cystitis and in pyelitis it is essential that the organism present in the infection be tested for its susceptibility to the bacteriophage to be employed. For purposes of therapy, that race of bacteriophage should be used which shows the highest

degree of virulence. Incidentally, it may be mentioned that sewage is a particularly good source for races of bacteriophage having a high virulence for organisms of this type.

The most satisfactory method of applying treatment involves bladder lavage, or the introduction of bacteriophage directly into the pelvis of the kidney, in conjunction with subcutaneous injection. In the case of subcutaneous administration, three injections of 2 cc. each may be given at 24-hour intervals. For instillation into the bladder 5 cc. of bacteriophage should be diluted in about 50 cc. of saline.

Experience has shown that it is also desirable to have the patient ingest, each morning before breakfast, 2 cc. of bacteriophage diluted in half a glass of water. This oral administration should be continued throughout the duration of the treatment or at least for a period of some 20 days. The intravesicular treatment can be continued indefinitely without causing any disturbance even though the offending organism is not sensitive to the bacteriophage given. The number of treatments will vary with the chronicity of the infection. From the results thus far reported it would seem that success attends bacteriophage therapy in these diseases in about 60 per cent of the cases when treated by subcutaneous injection and intravesicular instillation. When, however, bacteriophage is introduced directly into the pelvis of the kidney, the re-

sults are definitely better; 85 per cent of the cases show recovery.

The above statements are based upon the results obtained in the treatment of some hundreds of cases of all types. In acute conditions the results are better, approximately 100 per cent of the cases recovering, and the evidences of the effectiveness of the treatment are immediate. In the more chronic conditions treatment must be continued over a longer period of time.

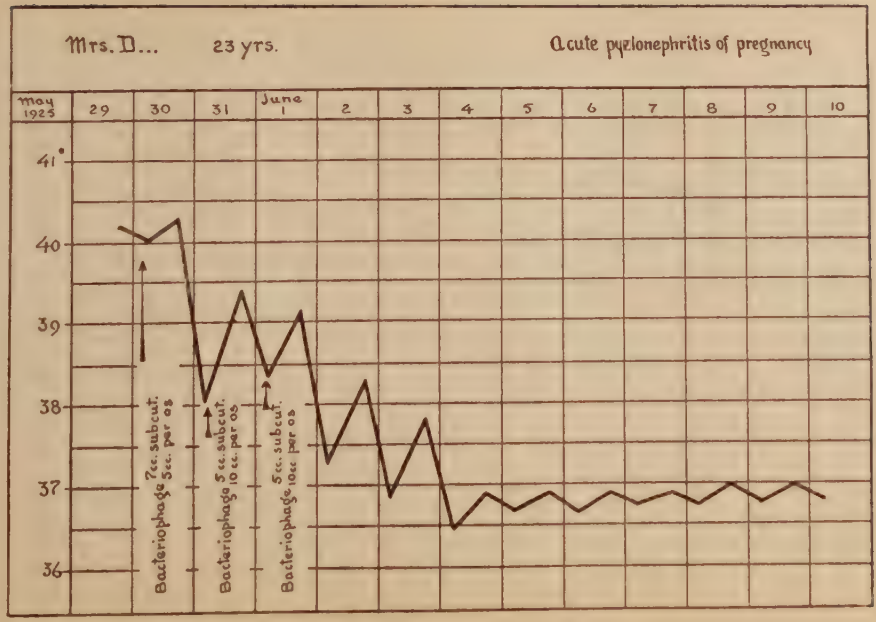
The many publications which have appeared leave no doubt as to the efficacy of bacteriophage in certain cases of infection of the urinary tract. Needless to say, the types of cases treated and the mode of application have varied widely. It is unnecessary to present examples of all types: only two will be given.

Mme. D....., aged 23 years, entered the hospital on May 29, 1925, with the diagnosis of acute pyelonephritis of pregnancy. She had been pregnant for 5 months. On May 21, general symptoms became apparent, with loss of appetite and weakness, but no fever. On May 23, she had a chill with an abrupt rise in temperature. Polyuria without dysuria developed. There was bilateral lumbar pain and the urine was definitely turbid. This condition continued, the temperature varying between 38 and 40°C. until her entrance into the hospital. At this time the temperature was 40.2°C. and on the following morning it was 40°C. Cultures obtained from a catheterized speci-

men of the bladder urine yielded an abundance of colon bacilli which subsequently proved to be susceptible to bacteriophagy. However, without awaiting the results of these tests, treatment with coli bacteriophage was instituted. On the 30th of May, 2 cc. were injected subcutaneously and 5 cc. were given by mouth. During the following evening the temperature rose to 40.3°C. On the 31st of May the morning temperature was 38.1°C., and a second treatment with bacteriophage was given, 5 cc. being injected subcutaneously and 10 cc. given by mouth. The evening temperature was 39.4°C. On June 1, with the temperature 38.4°C. in the morning, the bacteriophage treatment was repeated, the same doses being given as on the preceding day. The temperatures on June 2 were 37.3° in the morning, 38.3° in the evening, and the general condition was improved. On the following day the morning temperature was 36.9° and on June 4, with a temperature of 36.5°, the bladder symptoms had disappeared. The general condition was better, and the urine was less turbid but still yielded colon bacilli upon culture. On the 10th of June the patient left the hospital. In this case clinical recovery occurred, although from the bacteriological standpoint recovery was not complete.

A second case, one illustrative of chronic colon bacillus infection, may be given.

Mr. T....., 20 years of age, entered the hos-


Mrs. D... 23 yrs.
Acute pyelonephritis of pregnancy
May 1923
29
30
31
June 1
2
3
4
5
6
7
8
9
10
41°
40
39
38
37
36
Bacteriophage 7cc. subcut. 5cc. per os
Bacteriophage 5cc. subcut. 10cc. per os
Bacteriophage 5cc. subcut. 10cc. per os

pital on the 19th of May with the symptoms of a chronic colon bacillus pyelonephritis. The infection dated back to September, 1924, when evidences of a cystitis became apparent. Cystoscopy, in November of that year, confirmed the diagnosis and by catherization it was found that both kidneys were infected with the colon bacillus. From that time until entrance into the hospital various methods of treatment had been applied without success. Examination in the hospital revealed infection of the kidneys as well as a cystitis. The colon bacilli recovered in culture proved susceptible to bacteriophage. On May 20, 3 cc. of bacteriophage were injected subcutaneously and 5 cc. were given by mouth. On the 22nd a second injection of 3 cc. was given, and on the 28th this same dose was again repeated together with an instillation into the bladder of 10 cc. of bacteriophage. Specimens of the bladder urine collected later on the same day were sterile, as were specimens examined on June 5. In this case recovery, both clinical and bacteriological, occurred.

As has already been stated, it is not yet possible to treat *B. coli* infections with a "stock" bacteriophage. This fact limits the treatment to some extent to hospitals and to patients who can afford the preparation of an "auto-bacteriophage" by a specialized laboratory. Thus far a "panvirulent" race of bacteriophage, one active upon all strains of *B. coli,* has not been iso-

lated. Such races may be difficult to find, for those infections which become chronic are due to those bacterial strains which resist bacteriophage.

The whole question becomes singularly more complicated by the fact (probably this situation extends to all chronic diseases for it must represent a general condition, although as yet it has not been studied) that the organism isolated from the urine of the chronic case is not "the colon bacillus" as it is called in the text-book, but a variety or, indeed, often several varieties simultaneously present, of *B. coli,* each of which presents distinctive characteristics. As an example, I may present the data derived from a case recently studied. The complex nature of the problem becomes apparent at once.

An elderly man had suffered from cystitis for more than 30 years, although during this period his general health had been good. From his urine, five varieties of *B. coli* were isolated, and each variety showed clear-cut characteristics as appears in the following table.

The susceptibility of these diverse varieties of *B. coli* for diverse races of bacteriophage varied very greatly. Numbers 1 and 2 were susceptible to several laboratory preparations of bacteriophage; Nos. 3, 4, and 5 were resistant. From several specimens of Paris sewage three races of active bacteriophage were isolated. The first of these showed a virulence for cul-

Strain	Colony type	Morphology	Gram staining	Fermentation Reactions							
				Glucose	Lactose	Saccharose	Maltose	Mannite	Dulcite	Dextrin	Inulin
1.	large, smooth	bacilli	negative	A*	A	—	A	A	A	A	A
2.	large, rough	bacilli	negative	AG	AG	—	AG	AG	AG	AG	AG
3.	viscous	bacilli	negative	A	A	A	A	A	—	A	—
4.	minute, transparent	cocci and bacilli, mixed.	cocci, neg. and pos; bacilli, neg. and pos.	AG	AG	—	AG	AG	AG	AG	AG
5.	very minute, opaque	cocci	pos.	A	AG	—	AG	AG	—	AG	—

A = acid production; AG = both acid and gas produced; — = no fermentation.

ture strains 1, 2, and 4; the second for 1, 2, and 3, and the third for 2, 4, and 5. A mixture of the three races of bacteriophage caused a complete dissolution of a mixture of the five varieties of *B. coli* recovered from the urine of the patient.

As this example shows, the preparation of an "autobacteriophage" may be very complicated. Furthermore, it may be added that the case mentioned above is in no way an exception. Such a situation is the one usually encountered in chronic cases.

Sprue

Much has been written concerning the etiology of sprue, and in view of the very divergent opinions expressed it may well be admitted that bacteriophage treatment of the disease must rest upon a rather insecure basis. Nevertheless, upon the strength of some as yet unpublished work done by Col. Morison and Major de Martin of the Indian Medical Service, it would appear that the causative, or at least contributory, agent of this disease is a paradysentery bacillus, which, because of its resistance to bacteriophage, has acquired modified cultural characteristics. But however this may be, bacteriophage treatment has been applied to 23 patients who had had sprue for periods ranging from 2 to 15 years. The bacteriophage employed was a stock antidysentery race, which had been adapted to the Flexner and Hiss

strains of *B. dysenteriae*. The mode of administration was as follows: three subcutaneous injections of 2 cc. each on three consecutive days, together with the daily ingestion before breakfast of 2 cc. of the bacteriophage preparation diluted in half a glass of water.

Of 8 similar cases which I have treated 7 were cured within less than 10 days, the only failure being in a man, an European 72 years of age, who had been sick for more than 20 years and who was in the final stage of the disease.

If we summarize, then, the results thus far obtained with bacteriophage therapy in infections of the enteric tract and in those due to organisms of the enteric group, we may say that this mode of therapy:—

1. Is specific for bacillary dystentery.
2. Is specific for Asiatic cholera.
3. Is of little, if any, value in the typhoid and paratyphoid fevers.
4. Is highly effective in acute colon bacillus infections; somewhat less valuable in chronic conditions.
5. Offers much promise in infantile diarrheal conditions and in enterocolitis.
6. Upon the basis of a limited experience appears effective in sprue.

Infections Due to the Pyogenic Cocci.

Staphylococcus Infections

The situation with regard to staphylococcus infections and their treatment resembles in many respects that encountered in infections due to *B. coli.* Like the colon bacillus, the staphylococcus is of the heterogenic type and exhibits a considerable degree of strain specificity as regards susceptibility to bacteriophage, but while in principle the resemblance of the two species is close, in practice, procedure is simplified by the fact that a polyvirulent race of bacteriophage, race *h* of Gratia, has been found which apparently is effective with all susceptible strains of the staphylococcus. Since the discovery of this race it has been widely used in therapy and appears to have given results as satisfactory as those obtained with more highly strain-specific races. Staphylococcus infections of various types have been treated in one way or another with results that are quite generally satisfactory.

Furunculosis

Of all types of infection due to the staphylococcus, furunculosis has, undoubtedly, received the most attention from the standpoint of bacteriophage therapy. In a large part of the studies, the bacteriophage has been administered by subcutaneous injection, although a few investigators have combined such injections with the local application of bacteriophage in the form of moist dressings.

Usually two injections, each of which may vary from 0.5 cc. to 3 cc., have been given with an interval of from 24 to 48 hours.

While many authors have published the results of their use of bacteriophage in furunculosis, only one such publication need here be mentioned, that of Larkum, since in every large series of cases reported the results have in general been much the same. As reported by him the polyvirulent strain of bacteriophage was administered by subcutaneous injection, two doses of 2 cc. each being given on two successive days. In his summary, based upon 66 individuals treated, the cases are divided into two groups, those with chronic furunculosis (32), and cases in which the duration of the infection was not certainly ascertained although in some of them it surely was of long-standing (34). Of the 66 patients so treated, recovery or marked improvement occurred in all but one case and in the majority of instances there has been no evidence of a recurrence of the infection. Many other reports present results comparable in all respects to those of Larkum and, if due consideration be given to the variable conditions under which treatment has been applied, it can only be concluded that bacteriophage therapy in furunculosis is highly effective. It is perhaps significant that the point of injection appeared to be of little, if any, importance.

Another infectious process to receive attention from

Carbunculosis

the point of view of the practical use of bacteriophage is the so-called carbuncle, and while the experience here has been less extensive than in the case of furunculosis, the results obtained leave no doubt as to its effectiveness.

The results secured in a group of 48 cases has been summarized by Raiga, Chief of the Surgical Clinic on the Service of Professor Gosset of Paris. In his experience the results were extremely satisfactory.

In connection with this study some extremely interesting and important observations have been made which have a direct bearing upon bacteriophage therapy and deserve passing mention. It was observed in certain cases where the bacteriophage proved to be ineffective that the serum of the patient exhibited a definitely antibacteriophagic property. Further study of this phenomenon revealed the surprising fact that by means of autohemotherapy this antibacteriophagic property could be held in abeyance, or would disappear, with the result that through combined autohemotherapy and bacteriophage treatment the method is almost uniformly successful.

In this most successfully treated series of cases the bacteriophage was administered by direct injection into the carbuncle, and not only was ultimate recovery recorded, but improvement in the condition became apparent very quickly after the administration of bacteriophage, and healing was rapid. The large

scars which are so commonly found in association with healed infections of this type were lacking. The mode of bacteriophage treatment adopted by Raiga comprised from one to three subcutaneous injections together with injections locally into the lesion. This in turn was followed by the utilization of dressings moistened with bacteriophage. In giving the injection of bacteriophage directly into the lesion effort was made to effect a wide distribution of bacteriophage throughout the purulent material. After inserting the needle and expelling a portion of the bacteriophage, some of the material was drawn back into the syringe and then re-expelled. This process was repeated several times and in this way a more general contact of bacteriophage with the infecting organisms in different portions of the focus was effected. The subcutaneous injections, each of 2 cc., were given every other day. Injection into the purulent foci may be painful, but the physician may always resort to local anesthesia.

An incidental point may be mentioned although it is of greater importance to the patient than to the physician. The introduction of bacteriophage *in situ* exerts an effect upon the painfulness of the lesion. Very frequently within a few minutes after the instillation of bacteriophage into the lesion the pain disappears completely. A case cited by Raiga illustrates this action. Bacteriophage was administered locally to an elderly woman suffering from an anal abscess which

treatment. The latter was of two types. In the first cases treated, dressings moistened with bacteriophage were applied; the later cases were given injections directly into the lesion, and while this method caused at the time of injection considerable pain, beneficial results appeared somewhat more promptly. Local surgical treatment was not used.

In many cases the effect upon the purulent process was almost immediate, i.e., within 24 hours, and in 88 per cent purulation had ceased by the sixth day. Cure was prompt—42 of the 50 cases were completely healed within less than three weeks—but was determined to some degree by the location of the lesion. The more superficial infections responded more promptly, but even in the 25 cases in which the deeper tissues were involved the treatment was highly effective. In most cases the infection had been present for at least one week before treatment was started, and the single case which did not respond was one in which there was an osteitis and in whom the serum exhibited marked antibacteriophagic properties for both staphylococcus and streptococcus bacteriophage.

Acne

Several cases of acne, all of which had been treated previously by all known methods, including autogenous bacterial vaccines, have been treated by local applications of the antipyogenic bacteriophage. Within a period of from two to three weeks recovery was complete in each instance.

As an example, the following case may be cited. A young woman, a bank clerk, 30 years of age, had acne pustules localized on the face and neck. For a period of two years these had resisted all forms of treatment. The infection became so severe that she was taken from the window and put to work where she would not be required to meet the public. She treated herself, in accordance with advice given her, by scraping each pustule and then touching the lesion with cotton which had been dipped into a bacteriophage suspension. A month later the pustules had all disappeared.

In view of the claim that acne is caused solely by an anaerobic bacterium, *B. acne,* these results may raise a question. Whatever the fact may be regarding etiology, the fact remains that the disease may be cured by means of the antipyogenic bacteriophage suspension. It is possible that the bacteriophage in question adapts itself to the specific agent of the disease, or it may be that pyogenic organisms are accessory to the lesion, and once they are eliminated by means of bacteriophage the anaerobe is disposed of by other agencies.

In this connection it may be recalled that Gougerot obtained similar results in sycosis treated with antistaphylococcus bacteriophage, although up to now we have assumed another agent to be the cause of this disease.

The use of bacteriophage in treating osteomyelitis

has been decidedly limited, at least, published results are few, but from the few reports that have appeared it would seem that this mode of therapy offers very definite possibilities. The results can best be indicated by offering three examples of cases in which bacteriophage has been used; the first by Marceron, Chief of Clinic at the Saint Louis Hospital, the other two by Claeys and Peyre.

Osteomyelitis

Case 1. Miss Jar. . . . . , 22 years old, appeared at St. Louis Hospital on December 28, 1927, with cutaneous tuberculosis lesions on the outer third of the right leg. The disease began early in 1925, when the patient showed an indurated erythema which had become transformed into an ulcerous tuberculid. She was treated at the hospital by rest and tuberculin injections. A relapse occurred in June, 1927, and she was treated without success by the tuberculosis vaccine of Vaudremer. She was again sent to the hospital on December 28, 1927, for light treatment. The results represented an absolute failure.

During the treatment, the ulcerations increased and became infected; they oozed serum and suppurated abundantly. The bacterial flora showed a marked secondary infection, with enormous numbers of staphylococci. On May 10, the ulcerations had reached their maximum development. There was a large area, 13 by 10 cm., of erythema with numerous ulcerations varying in size from that of a lentil to that of a half-

dollar. On this date the first wet dressings containing a mixed antipyogenic bacteriophage preparation were applied. Cultures made on May 11 showed that the pus contained an extremely varied flora, with the staphylococcus, streptococci, and *B. coli* predominating. The odor of the wound was very offensive. A second wet dressing was applied on May 12. The pus was still abundant, and the erythematous area was of a brighter red color. The excoriations seemed to have deepened. Wet dressings saturated with bacteriophage were renewed every day, and improvement began on May 18, as was evidenced by the changed odor, the less abundant suppuration, and the appearance of small areas of new granulation tissue in the lesions. The dressings were now made at intervals of four days, and were moistened with a low dilution of bacteriophage in physiological salt solution. On June 14, the general aspect was much improved, there was a marked tendency towards healing, but, although much less abundant, the pus persisted. On June 18 the condition of the patient was completely changed. The pus was replaced by an abundant serous exudate which contained only an occasional staphylococcus and the lesions were in full process of regeneration. The erythema had become reduced and had changed from a dark red to a violet hue. The lesions became completely healed by the end of June.

Case 2. A young man, 26 years old, was seen in

November, 1924, for an inflammatory condition superimposed upon an old osteomyelitis of the left femur. This infection dated back 12 years, and for it the patient had been operated upon 14 times since 1912. There was a fistula leading in between the femoral condyles. The general health of the individual was profoundly affected. There was albumin in the urine and for several months there had been edema of the extremities.

On December 9, a specimen of the pus yielded a pure culture of *Staphylococcus aureus.* On December 11, the patient was operated upon, the infected area being opened and drainage established, and 2 cc. of bacteriophage suspension were poured into the lesion, which was afterward packed with gauze. Bacteriophage was applied repeatedly for four days. The temperature dropped to normal. On February 28, the patient left the hospital greatly improved, and when seen again in February, 1926, was found to be completely cured.

The third case, although perhaps less spectacular, is presented because it deals with that type of osteomyelitis, so commonly encountered in children, consequent to trauma.

The child, aged 8½ years, received, on January 9, an injury which, although painful, caused no concern until January 24, when the temperature suddenly rose to 39.5°C. When first seen in the hospital on Janu-

ary 27, a local inflammatory reaction was obvious. This, when aspirated, yielded 20 cc. of pus which gave, upon culture, a typical *Staphylococcus aureus*. Roentgenologic examination indicated an osteomyelitis.

A bacteriophage was adapted to the coccus isolated from the patient, and when the lesion was opened on January 31, 2 cc. of the bacteriophage were introduced. On the fourth day the temperature fell to 37°C. Dressings moistened with bacteriophage were applied on February 6, 9, 12, 16, and 20. On March 10, the lesion had completely healed.

While, of course, it is unwise to draw broad conclusions too freely, upon the basis of results of this character it is certainly permissible to indulge in the hope that infections of this type, so difficult to handle by other methods, will respond quite generally to bacteriophage therapy. Many hospitals in Paris are now employing bacteriophage in all types of suppurative processes and the results thus far have been most promising. In some institutions it has become the routine practise to utilize bacteriophage in connection with all operative procedures that involve entering the peritoneum.

Otitis

Several investigators have sought to treat otitis by means of the local application of bacteriophage, and opinions as to its value differ. That infections of this type do not uniformly respond to the treatment is unquestioned; indeed, some of

those who have been most favorably impressed with the treatment record cases in which the results were nil. It should be pointed out, however, that in these cases it has been found that the strains of staphylococci involved are of the resistant type.

Perhaps the largest series of cases to be reported by one person is that of Camus. His report embraces 2 cases of middle ear infection and 37 cases of infection of the external meatus. In all cases the bacteriophage was given solely by external local application.

Of the two cases of middle ear involvement, one responded to the treatment, one did not; but of the 37 cases of infection of the external ear 31 recovered within from two to four days.

Other Infections

As has been indicated in connection with acne, the use of bacteriophage active for pyogenic organisms may not be limited strictly to those infections in which the staphylococcus is the sole etiological agent. This organism may appear as an agent of secondary invasion under a variety of conditions, and through its mere presence in association with some specific etiology, may markedly aggravate the condition or cause it to be prolonged unduly.

Such, for example, seems to be the case in purulent infection superimposed upon psoriasis. It may be worth while to mention a case of this type in which

bacteriophage therapy has been applied with benefit.

A child, 5 years of age, because of the severe irritation, due to scratching, caused a secondary pyogenic infection of such extent that he presented, on June 17, suppurating areas of the anterior surfaces of both legs, of the sacro-lumbar region, and of the palmar surfaces of the right hand, of which the fingers were in flexion because of the pain produced on extension.

On that date a subcutaneous injection of 1 cc. of staphylococcus bacteriophage was given, and this was repeated the following day. He also was given a bath in 40 liters of boiled water to which a liter of antipyogenic bacteriophage had been added. In addition, he received daily local dressings with gauze soaked in bacteriophage. On June 25, his condition was greatly improved. On July 15, all suppuration had disappeared and at present his immunity to pyogenic organisms is such that scratching or other injuries fail to induce an infection of any kind.

Streptococcus Infections

The use of bacteriophage in streptococcus infections has not been as extensive as in the case of pyogenic processes associated with the staphylococcus. For this there are several reasons. In the first place, races of bacteriophage active for streptococci are more difficult to obtain, and the diversity of streptococcus strains renders the situation more complex. Further-

more, purely streptococcal infections are perhaps less clearly defined and all too frequently run an acute fulminating course, affording less opportunity for instituting treatment. On the other hand, those infections, such as endocarditis, which are exceptionally chronic conditions are invariably due to strains of the streptococcus of the resistant type.

The streptococcus appears to assume the bacteriophage-resistant form with relative ease, and as a species streptococcus is markedly heterogenic.

Nevertheless, in spite of these difficulties, streptococcus bacteriophage has been employed clinically in a variety of infectious conditions with considerable success. In this connection mention should be made of the work of McKinley, to which attention has already been called in The Bacteriophage and Its Behavior, and that of Dutton.

The observation of McKinley that streptococcal empyema could be effectively treated with bacteriophage has been supplemented by studies made by a number of investigators, although no one has recorded a case of greater interest than that originally presented by McKinley. The essential points of this case may be restated here, since they illustrate the features worthy of consideration.

The patient, a man aged 39 years, was first seen on March 9, having been ill for three weeks. Roentgenologic examination disclosed an abscess of the

lower right lung, and there was fluid in the chest. Examination showed that streptococci were present in this fluid. On March 13, a rib was resected and drainage was established. On the days following the operation relatively large amounts of pus having a particularly foul odor were obtained. On the 17th, 30 cc. of streptococcus bacteriophage were injected into the cavity, and this treatment was repeated on the 20th, although in the meantime the character and amount of the discharge had changed. By March 23, the discharge had ceased, but a third treatment with 25 cc. of bacteriophage was given. Two days later, on March 25, the patient was discharged from the hospital.

Streptococcus Septicemia

Dutton has given particular attention to the relationship of streptococcus to bacteriophage, correlating not only the cultural characteristics of the organisms with sensitivity to bacteriophage action, but also virulence factors, as indicated by the characteristics of the infectious process, with the way in which the organisms react to bacteriophage and the manner in which the infection responds to bacteriophage therapy.

Upon the basis of his studies, he divides streptococcus infections into three groups: Group 1, very mild infections characterized by spontaneous recovery; Group 2, somewhat more severe processes with recovery the usual outcome if complicating factors do not

intervene; and, Group 3, infections which are fatal despite all therapeutic procedures. The organism responsible for those infectious processes belonging to the first group are susceptible to the bacteriophage; those causing infections of group 2 may be either susceptible or resistant, but in any case are organisms admixed with bacteriophage; while those of group 3, causing fatal infections, are endowed with a high resistance. It is interesting that the first group frequently presents a septicemia, that the second group are chiefly localized processes, and that the third group represents those of the fatal septicemic type.

Incidental to his study, Dutton made practical application of bacteriophage therapy in certain cases of streptococcus septicemia. One such case may be mentioned since it shows not only the result of this therapy, but indicates as well the possibilities of applying bacteriophage by the intravenous route. This case was a puerperal septicemia due to the streptococcus, unmixed with bacteriophage, and naturally susceptible to its action. Bacteriophage in 1 cc. quantities was injected intravenously, three injections being given. Within 24 hours of the time of treatment the blood cultures were sterile, and recovery, although slow, was uneventful.

As has been stated above, all atttempts at treating *Streptococcus viridans* endocarditis have been complete failures, and the work of Dutton shows that in

infections of this type bacteriophage-resistant strains are invariably present.

Chronic Bronchitis

Just as antidysentery bacteriophage and antistaphylococcus bacteriophage appear to be effective in treating certain infections of uncertain etiology, such as sprue and acne—possibly through some mechanism as yet entirely unknown—so also antistreptococcus bacteriophage has found an application in infections of the upper respiratory tract, despite the fact that etiology is far from being established. While it must be admitted that in these conditions bacteriophage therapy can not yet be considered as an established procedure, in the sense in which it must now be regarded in bacillary dysentery, cholera, and certain types of *B. coli* and staphylococcus infection, the results thus far obtained deserve consideration.

In all of the cases of these types, bacteriophage preparations composed of a mixture of races, active for the organisms of the upper respiratory tract were used, the applications being made by thoroughly spraying the nares and posterior nasopharynx by means of an atomizer.

On November 7, 1927, a patient who had complained of a persistent bronchitis for a period of three months was given several treatments of the nose and mouth. Within 48 hours the patient felt more comfortable and breathed more freely; the bronchial râles

had almost entirely disappeared. On the 4th day the patient had fully recovered, and since then has had no further trouble.

Forty-one cases of bronchitis and nasopharyngeal catarrh have since been treated by Dr. Frumusan in this manner. Of these, 11 cases were completely cured, 5 showed a distinct improvement, and the balance failed to respond.

Angina

A case of bilateral angina, in a patient 22 years of age, was treated by Raiga, on September 12, 1928. When first seen the temperature was 39.3° C. and the patient was given a treatment, by means of an atomizer, with the bacteriophage. In addition, the patient used a solution containing bacteriophage as a gargle. At night the temperature was 38.6° C., and a subcutaneous injection of 2 cc. was given. The local treatment was continued on the 13th. On the 14th the temperature was 37.2°C. and the condition had improved. That night a second injection was administered. On the 15th recovery was complete.

Thirty additional cases of angina have since been treated in this fashion and in 60 per cent of them the effectiveness of the treatment was unquestioned.

Coryza

These results, obtained in bronchitis and in angina, suggested that bacteriophage therapy might find a possible application in coryza, although from the standpoint of specific etiology there would seem to be but little logic in such a course.

It is needless to repeat that the causative agent of this condition has never been identified and that there is great divergence of opinion as to the rôles of streptococci, filter-passing viruses, etc. in the disease. It is quite possible, indeed probable, that the clinical picture seen in an attack of coryza is not referable to but a single invading microorganism, and there is much justification for the belief that the disturbance caused by the primary invader—the filter-passing virus—is but transitory and in itself of minor significance. Its chief importance lies in the fact that associated with this initial invasion there is a marked depression of the normal resistance of the mucous membranes. This heightened susceptibility to invasion may not continue for more than 24 to 36 hours, but is of sufficient degree and duration to enable other bacteria normally found in the respiratory tract, streptococci in particular, to gain a foothold and these in turn act as incitants to the more persistent clinical features of the disease. Thus, while bacteriophage could hardly exert any effect on the filter-passing virus, or indeed, be in any way influential in combating the lesions caused by this agent, it is conceivable that by preventing the development of the organisms of secondary invasion it might transform what is so frequently a long drawn-out condition into one of short duration and of relatively minor importance.

With these ideas in mind a few cases were treated

with mixed streptococcus bacteriophage, the preparation being administered to the upper respiratory tract by means of an atomizer, and by the instillation of a few drops into the internal angle of each eye where ocular involvement was present. In some of the cases so treated the course of the disease was very definitely altered in that the whole evolution of the process was markedly accelerated and the entire disturbance had disappeared within from 36 to 48 hours.

In June, 1928, an opportunity presented itself to try the method in a mild epidemic of coryza. A series of 35 cases were treated. In none of the treated cases did the disturbance persist for more than 48 hours, while in the untreated control cases, some of whom were members of the same families as those treated, the disease ran its usual course, lasting for from 8 to 10 days. During the past winter about 100 additional cases have been given this mode of therapy, and in 70 per cent of them the results were very definitely beneficial.

Thus far has developed bacteriophage therapy with respect to pyogenic infections due to staphylococci and streptococci. Manifestly, in these diverse infections the procedure from the purely technical point of view is susceptible to improvement. The various types of infectious process, and the structure and distinctive features of the various regions in which the infection localizes may well demand distinctive methods of ap-

plication. Indeed, it is rather surprising to find that the results have been so favorable in view of the somewhat arbitrary procedures followed. However this may be, it is obvious that pyogenic infection, like enteric infection, offers a field for further study.

In summarizing the results of bacteriophage therapy of infections due to pyogenic cocci, or infections with which these organisms are associated, the following statements may be made:

1. The method is highly effective in treating furunculosis.

2. Applied by direct injection into carbuncles excellent results are obtained.

3. Almost uniformly successful results are secured in the treatment of paronychia.

4. Acne, at least in some cases, is benefited by bacteriophage treatment, as is sycosis.

5. Apparently the method can be successfully used in osteomyelitis.

6. Pyogenic infections of the ear, particularly those of the external ear, respond to the treatment, as does pyogenic infection superimposed upon psoriasis.

7. Wounds infected with pyogenic cocci may be treated to advantage.

8. The method as at present applied is apparently devoid of value in streptococcal endocarditis; in some other types of septicemia, where the streptococcus involved is not of the resistant type, the treatment is effective.

9. Upon the basis of limited experience it would seem that streptococcus empyema is amenable to bacteriophage therapy.

10. Promising results have been obtained in chronic bronchitis, in angina, and in coryza.

Bubonic Plague

Brief consideration should be given to *B. pestis* infection as seen in plague of the bubonic type, since this organism possesses little in common with those already discussed and the general characteristics of the infectious process differ materially from those associated with either the enteric or pyogenic groups of bacteria.

Unfortunately, up to the present time, bacteriophage therapy of bubonic plague has not been undertaken upon a scale sufficient to justify a final conclusion as to its general value, but if it is permissible to judge from the studies now available it can be concluded that the method offers much promise. In 1926, in The Bacteriophage and Its Behavior, I reported on the treatment of four cases. One such case will be quoted:

"Case 3. Theodoro Cass..... cabin-boy, 16 years old.

"On the 12th of July he presented the symptoms of a febrile gastric disturbance, and on this same day he

was isolated in the hospital. His condition grew worse on the evening of the 14th.

"On the morning of the 15th the pulse was irregular at 126; the temperature was 39.4°C.; the conjunctivae were injected; and there was a marked prostration. During the night a swelling of the right submaxillary gland developed, it becoming the size of a hazelnut, and was painful when pressed.

"Material aspirated from the gland revealed coccobacilli in direct smears. Cultures from the material gave typical *B. pestis,* and a guinea pig inoculated with it died 56 hours later with all of the lesions characteristic of experimental plague in this animal.

"On the 15th, at 3 P.M., I injected 1 cc. of the bacteriophage suspension directly into the center of the swollen gland.

"On the morning of the 16th, all of the symptoms, with the exception of the bubo, had disappeared. The patient was lively, and was sitting up in bed when I made my visit. His temperature was 37.2°C.; the pulse 70. He stated that he had felt no ill effects as a result of the injection and that he had slept well. I had requested the attendants to watch him closely throughout the night and they confirmed his statement —they had observed no evidence of a reaction, no sweating and no restlessness. A few hours after the injection the patient had told them that he felt better and that he was going to sleep. When he woke up he told them that he was well.

"Considering the nature of the disease, and the serious condition of the three patients, I had feared that there might be a marked general reaction as a result of the *in vivo* bacteriophagy of the plague bacilli. But absolutely nothing of the kind occurred. There was not even a local reaction, for 16 hours after the injection, in all cases, the bubo was less painful.

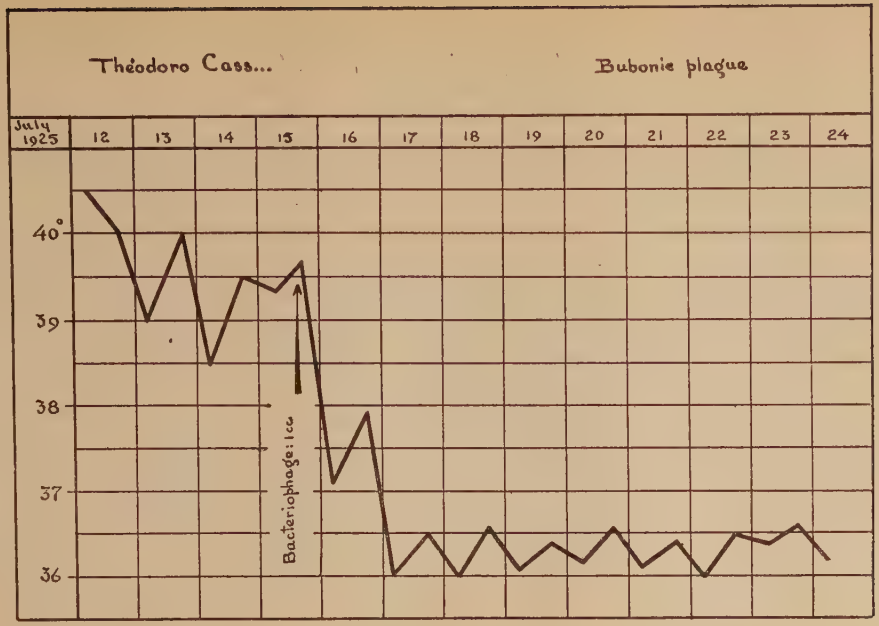
"On the morning of the 18th, as with the preceding cases, 60 hours after the inoculation, I withdrew some of the material from the gland. Direct examination, culture, and guinea pig inoculation, all showed that the contents of the bubo were sterile. A suspension of plague bacilli, added to the bouillon which had shown no growth, failed to develop and were completely bacteriophaged in 7 hours.

"The bubo absorbed slowly. On the 8th of August it was still as large as a pea."

More recently these results have been confirmed by Doorenbos at Suez, where he has treated 9 cases in a similar manner with but one death.

While these results can be considered only in the light of preliminary studies, they are sufficiently suggestive to warrant further study, and fortunately such studies are now actively being pursued to the end of establishing just how effective this mode of therapy may be in this disease.

In discussing the subject of bacteriophage therapy it may not be out of place to state that the possibilities


Théodoro Cass...
Bubonic plague
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Bacteriophage: 1 cc

of bacteriophage treatment are not of necessity restricted to the infectious processes here discussed. Indeed, races of bacteriophage have been isolated which are active upon a variety of other organisms and it may well be that with the development of additional methods, and in particular, with the isolation or adaptation of highly potent preparations many new fields of use will become apparent.

While not particularly pertinent to a discussion of the treatment of human infections, it is not without interest to recall that results of exactly the same nature have been obtained through the use of bacteriophage in various animal diseases. The diseases, in which therapy has proved effective, are of very diverse natures and are infections occurring in very different species of animal. A detailed discussion of these results is not called for here; those interested may find these infections discussed at length in the texts already mentioned.

Another related subject may be mentioned. The above discussion of the relationship of bacteriophage to infectious disease from the standpoint of its direct application has dealt entirely with the therapeutic aspect of the subject. As has been stated above, bacteriophage as now prepared, represents not only a suspension of bacteriophage corpuscles but, among other components, the disintegrated material of the bacterial cells. In undergoing bacteriophagy, this ma-

terial has not lost its immunizing property, but, on the contrary, it would seem that through the action of bacteriophage it has been brought into such a state that it is more highly immunizing than when present in the intact bacterium. Reference need only be made to my early studies in connection with hemorrhagic septicemia in the water-buffalo and with fowl-typhoid. In these studies it was shown that the injection of bacteriophage preparation would confer, after a period of latency, a state of immunity adequate to protect the inoculated animal against several hundred, or even two thousand, lethal doses of the causative organism. These results have since been confirmed in the case of the water-buffalo, and the method has since been employed on a large scale throughout Indo-China. The results with fowl-typhoid have since been duplicated with fowl-cholera, and recently Compton has succeeded in protecting mice against *B. pestis* infection by the same method. Larkum, in Michigan, is conducting experiments of a similar nature in man through the use of bacteriophage preparations as an immunizing agent for human typhoid fever. Obviously, from the standpoint of inducing the development of a more lasting organic immunity these results are of importance and it is entirely possible that in the bacteriophaged suspensions of many types of bacteria, agents will be found suited to serve as vaccinating material.

In connection with the question of prophylaxis one interesting possibility may be mentioned, since it involves the prevention of disease through the operation of an exogenous rather than an endogenous immunity. It should be understood, however, that the procedure suggested is to be regarded solely as a working hypothesis.

We know that at the time of birth the intestinal tract of the infant is free of bacteriophage. Only after a period varying between four and ten days does bacteriophage make its appearance within the enteric tract, and the race of bacteriophage then implanted appears to be retained throughout the life of the individual. At the time of implantation, colon bacilli are also implanted and the symbiosis established between these bacilli and the bacteriophage persists. This race of bacteriophage, thus accidentally implanted, possesses its individual characteristics. It may be a race endowed with high virulence and a race which readily undergoes adaptation, or, on the other hand, it may be a race of low virulence or one which can be adapted to other organisms only with difficulty. Experiment and clinical studies have shown that the intestinal bacteriophage is of great importance in the natural processes of recovery. Obviously, when the race of bacteriophage present is one which readily undergoes adaptation the chances of spontaneous recovery, or, indeed, for overwhelming invading organ-

isms before the disease becomes manifest, are greatly increased. This suggests that instead of allowing accident to determine the qualities of the bacteriophage implanted in the infant, it might be advantageous to implant, soon after birth, a bacteriophage of known properties, both as regards its virulence and its power of adaptation. To do this, and to permit the establishment of a suitable symbiosis, an appropriate race of *B. coli* must also be implanted. Such a procedure could be of no possible danger and might very well be of considerable benefit.

In concluding this chapter it must be stated that bacteriophage therapy is only beginning. In but a few instances has it passed the experimental stage, but in these instances the outcome is not open to question.

Although the general principles which must govern bacteriophage therapy were announced in 1921, it is only during the past two or three years that attention has been seriously directed to the practical application of these principles. Today, there is hardly a country in which investigators—bacteriologists, and clinicians as well—may not be found endeavoring to extend the field and to gain additional knowledge concerning the phenomena involved.

Much remains to be accomplished. It is still necessary to isolate powerful races of bacteriophage for many of the pathogenic organisms. It is still necessary to study further the pathogenic characteristics of

the bacterium-bacteriophage symbiosis, and it is particularly essential that the best method of application be determined for each particular disease. Upon the basis of what has now been accomplished it might, with some assurance, be predicted that with added information and experience we will have in bacteriophage a mode of therapy for all infectious diseases of bacterial origin.

It is not without interest to recall that in 1890, von Siemens, the German inventor and philosopher, in writing his memoirs made a most significant statement with reference to the treatment of tuberculosis with tuberculin as then advocated by Koch. In substance, he stated that he did not believe that disease could ever be cured through the administration of products secreted by the pathogenic bacterium itself, but that specific therapy would be possible only when a natural enemy—a parasite—of the bacteria had been discovered. Apparently this scientist predicted truly.

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Conclusions

IT WOULD seem that after reading the pages of this text, in which it has been possible to present but a brief summary of those phenomena based upon bacteriophagy, there can hardly remain in the mind of the reader any doubts concerning the statements made in the preface. Under the impact of the ideas gradually revealed in the study of bacteriophagy the whole time-honored biological structure is shaking upon its foundations.

The first conclusion has a general biological bearing. The bacteriophage is a living being because it possesses all of the attributes of life, in particular, it has a metabolism which is its own and which, furthermore, operates as does the same phenomenon in all other living beings. In view of its size, this living being can only be formed of a simple protoplasmic micella. This, in itself, suffices to demonstrate that the cellular theory of life is false; that the cell is not the unit of life.

With what sort of a theory can we replace the cellular theory? The cell is an organism already of extreme complexity. Just as the body of a higher animal is formed of an assemblage of tissues, each tissue being composed of an assemblage of cells, so is the

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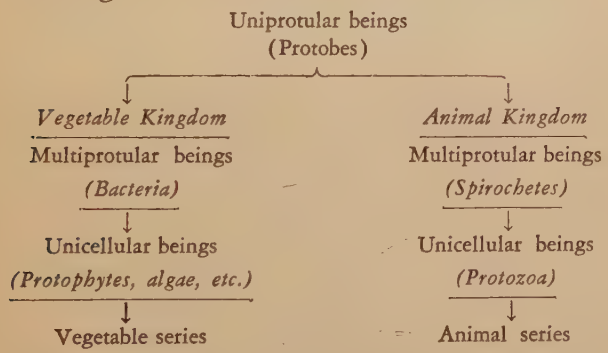
cell differentiated into nucleus and cytoplasm and each of these is constituted of an assemblage of "elementary micellae." Each micella is an autotrophic living entity, possessing its independent metabolism, reproducing, and transmitting its characters to its descendents. One might term these elementary micellae "Protules." Cellular metabolism thus becomes the sum of the elementary metabolic activities of those protules which through union form the complex structure of the cell. Just as the atom is not the unit of matter, so also the cell is not the unit of life.

What is the size of the protule? The bacteriophage, like several other infravisible viruses which experiment has shown to be of essentially the same dimensions, appears to be formed of but a simple protule. They have a diameter of between 30 and 35 millimicrons. It is of interest that geneticists have estimated the gene to be of about 35 millimicrons also. From this resemblance in size, certain authors have drawn the deduction that the bacteriophage is a gene, but such reasoning, based solely upon this fact, is as superficial as to conclude that a man is a shark because the man and the shark weigh the same number of pounds. All that may be said is that, in so far as we now know, it seems that the protule—the autonomous, elementary living particle which transmits its own characters to its descendents—has a diameter of about 35 millimicrons.

According to the cellular theory of life the cell was the unit of living substance. In reality, this theory was incompatible with the physico-chemical theory of life, for it is hard to conceive of the formation of an organism as complicated as the cell by a single act of physical forces. Such a formation can be comprehended only when we deal with undifferentiated elementary micellae. May I recall in this connection that I have demonstrated in natural sulfur-containing waters the presence of an infravisible virus endowed with the property of causing a reduction of sulfur compounds. This virus may have been the original living being. Possibly the first stages of the evolutionary scheme may have been in conformity with the following:



There has been much discussion as to the nature, animal or vegetable, of the filterable viruses (Protobes), for certain of their characters resemble those of bacteria, while others correspond to those of the

protozoa. This is but natural, for these beings are to be found as the basic form and are neither the one nor the other, or rather they are the one and the other, and it must be through a separation of the two that the two kingdoms are derived. We must, as a matter of fact, admit that the appearance of living beings on the earth must have been in the order of their complexity, the simplest forms appearing first, and what could have been more simple than a protobe, formed of but a single protoplasmic micella?

In the scheme here given the bacteria are classified as "multiprotular" beings in accord with a theory which I have been advancing for several years. There is, in fact, reason to believe that bacteria are not unicellular beings, that is, formed of a cell in the histological sense of the term, for they are not differentiated into nucleus and cytoplasm. We must not forget that the cell is but an expression of morphology, and that Schaudinn was guilty of real heresy when he advanced the hypothesis of a "diffuse nucleus." The nucleus is a morphologic entity and if the nuclear substance is disseminated throughout the whole being the matter of a nucleus can not come into question. The existence of infravisible, filterable, forms of bacteria (for which I have proposed the name "Protobacteria") suggests another hypothesis, in accordance with which the bacteria are plasmodia, formed of collections of protules, each protule

being by itself a living entity. In other words a bacterium is an assemblage of protobacteria, each protobacterium being formed of a protule. Differentiation into nucleus and cytoplasm, the essential character of the cell, can have taken place only later in the course of evolution, first in the algae which are the most simple cellular beings.

The second general conclusion deals with the matter of the fixity of species and with the rôle of symbiosis in the phenomenon of evolution.

Bacteriophagy is an infectious disease destructive to bacteria. The agent of this disease is a filterable virus, a protobe, the bacteriophage. When attacked by a bacteriophage having a very high virulence, the bacterium is destroyed, but if the attacking bacteriophage is of lower virulence, the bacterium is capable of resisting and it thus acquires a true immunity to its parasite. In brief, when bacteria and bacteriophage are brought together one of three things will happen in accordance with the conditions present. The bacteriophage may destroy the bacteria; the bacteria may resist and destroy the bacteriophage; or, finally, an equilibrium may become established between the resistance of the bacteria and the virulence of bacteriophage. In this last case, both survive and the bacteria contract a chronic disease, that is, a symbiosis is established. Such a state of equilibrium is, indeed, extremely frequent in nature.

Under the influence of the symbiosis the bacteria undergo profound modifications which may alter all of their characters. These modifications are, as we have seen, brought about through the loss or diminution, acquisition or increase of characters, each character varying quite independently of the others, as is characteristic for mutations. All of these mutations may be produced experimentally *in vitro*.

I have stated elsewhere* that there are reasons for believing that *all* sudden mutations occurring in nature are initiated by the state of symbiosis, and that, further, the slow adaptations to the environment and sudden mutations due to symbiosis are most probably the two phenomena which have governed the evolution of beings. The study of bacteriophagy permits us for the first time to study mutations experimentally and to ascertain their relationship to the general processes of evolution. As a corollary to this, is the fact that the possibility of inducing mutations at will shows clearly that the dogma of the fixity of species is contradicted by experiment.

This brief outline shows at once the importance of the phenomena of bacteriophagy from the point of view of general biology. Its importance is no less in its relations to pathology, immunology, epidemiology, and hygiene.

* Immunity in Natural Infectious Disease, Williams & Wilkins Co., Balt., 1923.

With reference to pathology, study of the phenomena of bacteriophagy leads to some very interesting conclusions. We have seen that symbiosis with a bacteriophage leads to profound modifications in the bacterium. One of the characters most readily influenced is virulence, which, as a rule, becomes attenuated or may even completely disappear. This loss in virulence is revealed not only through virulence *per se,* but such bacteria with a reduced or complete loss of virulence are no longer subject to phagocytosis, or, at least, they are phagocytized less readily. A result of these complex phenomena is that the acute diseases of man and of animals are caused by "ultrapure" bacteria, that is, by normal, healthy bacteria which have not been parasitized by the bacteriophage, while the chronic diseases, on the contrary, are due to the bacterium-bacteriophage symbiosis. In these chronic diseases the pathogenic bacteria are parasitized in a chronic manner by bacteriophage and their characteristics are so modified that they induce in the host a disease which is likewise chronic.

It must suffice merely to mention these facts which have received more extensive treatment elsewhere, but I may add that recent studies, as yet unpublished, show that the chronic disease par excellence, tuberculosis, must be caused by a bacterium-bacteriophage symbiosis. That which we term *B. tuberculosis* is the result of this symbiosis; the ultrapure bacterial form

presents completely different morphological, cultural, and biochemical properties.

A study of bacteriophagy *in vivo* overturns at once all of our older beliefs concerning immunity. Recovery from an infectious disease does not result, in reality, from a phenomenon of immunity; recovery is the direct result of bacteriophagy *in vivo* which leads to the destruction of the pathogenic bacteria, either directly, or indirectly through the potent opsonic action of the lysins secreted by the bacteriophage during its activity. The act of recovery is the result of the destruction of the pathogenic bacteria, but the action of the bacteriophage is not limited solely to this. During bacteriophagy *in vivo* the substance of the destroyed pathogenic bacteria becomes distributed throughout the body and the many experiments made in studying hemorrhagic septicemia in cattle have shown that these bacterial substances, rendered soluble by the ferments secreted by the bacteriophage, are in a physical or chemical state highly suited to stimulating the body in such a way as to lead to an acquired immunity. Basing their conclusions on these observations and upon their own experiments, Compton has recently advocated prophylactic vaccination for bubonic plague and Larkum has attempted vaccination against typhoid fever by means of the injection of bacteria dissolved *in vitro* by bacteriophage.

The action of the bacteriophage within the body is, therefore, very complex: it is the primary factor in recovery and it is the indirect agency of acquired immunity.

The revolution which the question of the bacteriophage has brought about within the realm of theory has its counterpart in the practical domain of therapeusis.

Recovery in infectious disease is the result of *in vivo* bacteriophagy, but the natural process is subject to change. It may be delayed in becoming established, when through the action of the pathogenic bacteria the organic lesions already produced are by themselves sufficient to cause death. Or, it may take place at a time, when, because of unfavorable circumstances, the natural process cannot be carried to completion. In such a case the pathogenic organisms develop without hindrance and the patient succumbs.

We have seen that the production of bacteriophage *in vitro* is readily accomplished. Why not administer selected cultures of bacteriophage of high virulence for the infecting bacteria at the very beginning of the invasion of the body by the pathogenic bacteria? Why not administer a virulent bacteriophage at the time of the first symptoms? This is precisely what I attempted first in bacillary dysentery, and later in many other diseases. The success attending the method has been disclosed in the preceding pages.

I may, however, state that therapy with bacteriophage provides the specific therapy par excellence and, it might be said, the only possible natural specific therapeusis, for it is the exact experimental reproduction of the natural process of recovery.

The influence of the question of the bacteriophage upon epidemiology and upon hygiene are no less profound. All of the theories advanced up to the present bearing upon the origin, the course, and the extinction of epidemics are effectively uprooted, for they failed to recognize the principal factor involved. The story of an epidemic is but the story of the struggle between the bacteria and their parasite, the bacteriophage. The phenomenon which most impresses us is the abnormal mortality which takes place in a community of men or of animals, but after all this represents only an epiphenomenon; the chief phenomenon is the struggle between the two infinitely small beings.

As may be seen, the question of the bacteriophage even has its philosophical aspect which is not without interest. But if the epidemic is, as a matter of fact, but an epiphenomenon, the man in whom it takes place may sometimes modify its course. He finds the bacteriophage as his ally, he may favor it, he may cultivate it artificially, he may distribute it throughout the environment, and by these means he may hasten the natural process leading to the extinction of epidemics.

Such, briefly stated, are the results of the study of bacteriophagy. I believe that I did not overstate matters in writing in the preface that the discovery of the bacteriophage was of such a nature as to cause within the biological sciences a revolution comparable to that which the discovery of the electron caused in the physical sciences.

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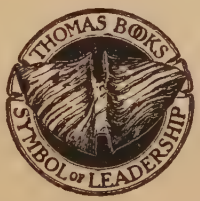
This Book

The Bacteriophage and Its Clinical Applications

was set, printed and bound by The Collegiate Press of Menasha, Wisconsin. The Cover Design is by the Decorative Designers of Chatham, New Jersey. The type face is Garamont. The type is set 12 on 14 point (Linotype). The type page is 20 x 35 picas. The end sheets are 23 x 35—95 (60 lb. basis) White Antique Buckeye. The Jacket is 24 x 36—80 Gray Antique construction. The text paper is White Antique Louvain Book 32 x 44—89 (60 lb. basis). The binding is Holliston Extra Colors T228.



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